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Selected scientific information through early 1985 is presented and evaluated relative to the health and welfare effects associated with exposure to ozone and other photochemical oxidants.

Data on health and welfare effects are emphasized; additional information is provided for understanding the oxidant pollution problem, for evaluating the reliability of effects data, and for assessing effects data relative to concentration occurring in ambient air. Information is also presented on exposure-related topics: sources, measurement, and concentrations of oxidant precursors; formation and transport of ozone and other photochemical oxidants; properties, chemistry, and measurement of ozone and other photochemical oxidants; and the concentrations of ozone and other photochemical oxidants typically found in ambient air.

The specific areas addressed by chapters on health and welfare effects are the toxicological appraisal of effects of ozone and other oxidants; effects observed in controlled human exposures; effects observed in field and epidemiological studies; and effects on vegetation, natural and agroecosystems, and nonbiological materials.

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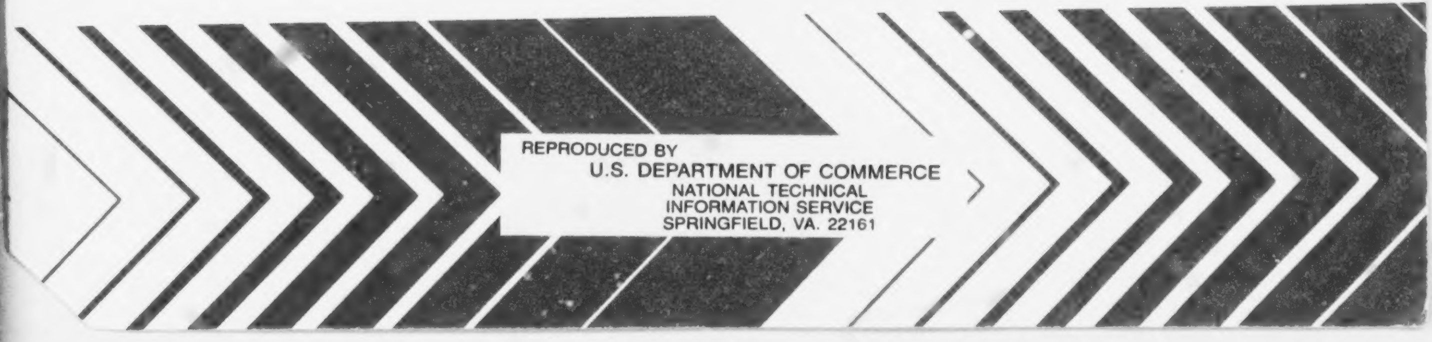

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Volume IV of V

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

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ABSTRACT

Scientific information is presented and evaluated relative to the health and welfare effects associated with exposure to ozone and other photochemical oxidants. Although it is not intended as a complete and detailed literature review, the document covers pertinent literature through early 1985.

Data on health and welfare effects are emphasized, but additional information is provided for understanding the nature of the oxidant pollution problem and for evaluating the reliability of effects data as well as their relevance to potential exposures to ozone and other oxidants at concentrations occurring in ambient air. Information is presented on the following exposure-related topics: nature, source, measurement, and concentrations of precursors to ozone and other photochemical oxidants; the formation of ozone and other photochemical oxidants and their transport once formed; the properties, chemistry, and measurement of ozone and other photochemical oxidants; and the concentrations of ozone and other photochemical oxidants that are typically found in ambient air.

The specific areas addressed by chapters on health and welfare effects are the toxicological appraisal of effects of ozone and other oxidants; effects observed in controlled human exposures; effects observed in field and epidemiological studies; effects on vegetation seen in field and controlled exposures; effects on natural and agroecosystems; and effects on nonbiological materials observed in field and chamber studies.

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LIST OF ABBREVIATIONS

A-V	Atrioventricular
ACh	Acetylcholine
AChE	Acetylcholinesterase
AM	Alveolar macrophage
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ATPS	ATPS condition (ambient temperature and pressure, saturated with water vapor)
BTPS	BTPS conditions (body temperature, barometric pressure, and saturated with water vapor)
CC	Closing capacity
C_{dyn}	Dynamic lung compliance
CHEM	Gas-phase chemiluminescence
C_L	Lung compliance
C_{Lst}	Static lung compliance
CMP	Cytidine monophosphate
CNS	Central nervous system
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COLD	Chronic obstructive lung disease
COMT	Catechol-o-methyl-transferase
CO_2	Carbon dioxide
CPK	Creatine phosphokinase
CV	Closing volume
D_L	Diffusing capacity of the lungs
D_LCO	Carbon monoxide diffusing capacity of the lungs
DNA	Deoxyribonucleic acid
E	Elastance
ECG, EKG	Electrocardiogram
EEG	Electroencephalogram
ERV	Expiratory reserve volume
FEF_{max}	The maximal forced expiratory flow achieved during an FVC test

LIST OF ABBREVIATIONS (continued)

FEF	Forced expiratory flow
$FEF_{200-1200}$	Mean forced expiratory flow between 200 ml and 1200 ml of the FVC [formerly called the maximum expiratory flow rate (MEFR)].
$FEF_{25-75\%}$	Mean forced expiratory flow during the middle half of the FVC [formerly called the maximum mid-expiratory flow rate (MMFR)].
$FEF_{75\%}$	Instanteous forced expiratory flow after 75% of the FVC has been exhaled.
FEV	Forced expiratory volume
FIVC	Forced inspiratory vital capacity
f_R	Respiratory frequency
FRC	Functional residual capacity
FVC	Forced vital capacity
G	Conductance
G-6-PD	Glucose-6-phosphate dehydrogenase
G_{aw}	Airway conductance
GMP	Guanosine monophosphate
GS-CHEM	Gas-solid chemiluminescence
GSH	Glutathione
GSSG	Glutathione disulfide
Hb	Hemoglobin
Hct	Hematocrit
HO·	Hydroxy radical
H_2O	Water
IC	Inspiratory capacity
IRV	Inspiratory reserve volume
IVC	Inspiratory vital capacity
$\bar{K}_o$	Average mucous production rate per unit area
LDH	Lactate deyhydrogenase
LD_{50}	Lethal dose (50 percent)
LM	Light microscopy
LPS	Lipopolysaccharide

LIST OF ABBREVIATIONS (continued)

MAO	Monamine oxidase
MAST	KI-coulometric (Mast meter)
max $\dot{V}_E$	Maximum ventilation
max $\dot{V}O_2$	Maximal aerobic capacity
MBC	Maximum breathing capacity
MEFR	Maximum expiratory flow rate
MEFV	Maximum expiratory flow-volume curve
MetHb	Methemoglobin
MMFR or MMEF	Maximum mid-expiratory flow rate
MNNG	N-methyl-N'-nitrosoguanidine
MPO	Myeloperoxidase
MVV	Maximum voluntary ventilation
NBKI	Neutral buffered potassium iodide
$(NH_4)_2SO_4$	Ammonium sulfate
NO_2	Nitrogen dioxide
NPSH	Non-protein sulfhydryls
O_2	Oxygen
O_2^-	Oxygen radical
O_3	Ozone
$P(A-a)O_2$	Alveolar-arterial oxygen pressure difference
PABA	Para-aminobenzoic acid
P_ACO_2	Alveolar partial pressure of carbon dioxide
$PaCO_2$	Arterial partial pressure of carbon dioxide
PAN	Peroxyacetyl nitrate
P_AO_2	Alveolar partial pressure of oxygen
PaO_2	Arterial partial pressure of oxygen
PEF	Peak expiratory flow
PEFV	Partial expiratory flow-volume curve
PG	Prostaglandin
pH_a	Arterial pH
PHA	Phytohemagglutinin
P_L	Transpulmonary pressure
PMN	Polymorphonuclear leukocyte
PPD	Purified protein derivative

LIST OF ABBREVIATIONS (continued)

P_{st}	Static transpulmonary pressure
PUFA	Polyunsaturated fatty acid
R	Resistance to flow
Raw	Airway resistance
RBCs	Red blood cells
R_{coll}	Collateral resistance
R_L	Total pulmonary resistance
RQ, R	Respiratory quotient
R_{ti}	Tissue resistance
RV	Residual volume
SaO_2	Arterial oxygen saturation
SCE	Sister chromatid exchange
Se	Selenium
SEM	Scanning electron microscopy
SGaw	Specific airway conductance
SH	Sulfhydryls
SOD	Superoxide dismutase
SO_2	Sulfur dioxide
SPF	Specific pathogen-free
SRaw	Specific airway resistance
STPD	STPD conditions (standard temperature and pressure, dry)
TEM	transmission electron microscopy
TGV	Thoracic gas volume
TIC	Trypsin inhibitor capacity
TLC	total lung capacity
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TV	Tidal volume
UFA	Unsaturated fatty acid
UMP	Uridine monophosphate
UV	Ultraviolet photometry
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_A/\dot{Q}$	Ventilation/perfusion ratio

LIST OF ABBREVIATIONS (continued)

VC	Vital capacity
$\dot{V}CO_2$	Carbon dioxide production
V_D	Physiological dead space
$\dot{V}_D$	Dead-space ventilation
$V_{D\ anat}$	Anatomical dead space
$\dot{V}_E$	Minute ventilation; expired volume per minute
$\dot{V}_I$	Inspired volume per minute
V_L	Lung volume
$\dot{V}_{max}$	Maximum expiratory flow
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_2$, $\dot{Q}O_2$	Oxygen consumption
^{125}I	Radioactive iodine
5-HT	5-hydroxytryptamine
6-P-GD	6-phosphogluconate dehydrogenase

MEASUREMENT ABBREVIATIONS

g	gram
hr/day	hours per day
kg	kilogram
kg-m/min	kilogram-meter/min
L/min	liters/min
ppm	parts per million
mg/kg	milligrams per kilogram
mg/m^3	milligrams per cubic meter
min	minute
ml	milliliter
mm	millimeter
$\mu g/m^3$	micrograms per cubic meter
µm	micrometers
µM	micromolar
sec	second

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chapter 9: Toxicological Effects of Ozone and Other Photochemical Oxidants

Principal Authors

Dr. Donald E. Gardner
Northrop Services, Inc.
Environmental Sciences
P.O. Box 12313
Research Triangle Park, NC 27709

Dr. Judith A. Graham
Health Effects Reearch Laboratory
MD-82
U.S. Environmental Protection Aency
Research Triangle Park, NC 27711

Dr. Susan M. Loscutoff
16768 154th Ave., S.E.
Renton, WA 98055

Dr. Daniel B. Menzel
Laboratory of Environmental Toxicology
and Pharmacology
Duke University Medical Center
P.O. Box 3813
Durham, NC 27710

Dr. Daniel L. Morgan
Laboratory of Environmental Toxicology
and Pharmacology
Duke University Medical Center
P.O. Box 3813
Durham, NC 27710

Dr. John H. Overton, Jr.
Health Effects Research Laboratory
MD-82
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Mr. James A. Raub
Environmental Criteria and
Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Stephen C. Strom
Department of Radiology
Duke University Medical Center
P.O. Box 3808
Durham, NC 27710

Dr. Walter S. Tyler
Department of Anatomy
School of Veterinary Medicine
University of California,
Davis, CA 95616

Contributing Authors

Mr. James R. Kawecki
TRC Environmental Consultants, Inc.
701 W. Broad Street
Falls Church, VA 22046

Dr. Frederick J. Miller
Health Effects Research Laboratory
MD-82
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Ms. Elaine D. Smolko
Laboratory of Environmental Toxicology
and Pharmacology
Duke University Medical Center
P.O. Box 3813
Durham, NC 27710

Dr. Jeffrey L. Tepper
Northrop Services, Inc.
Environmental Sciences
P.O. Box 12313
Research Triangle Park, NC 27709

Authors also reviewed individual sections of the chapter. The following additional persons reviewed this chapter at the request of the U.S. Environmental Protection Agency. The evaluations and conclusions contained herein, however, are not necessarily those of the reviewers.

Dr. Karim Ahmed
Natural Resources Defense Council
122 East 42nd Street
New York, NY 10168

Dr. Ann P. Autor
Department of Pathology
St. Paul's Hospital
University of British Columbia
Vancouver, British Columbia
Canada V6Z1Y6

Dr. David V. Bates
Department of Medicine
St. Paul's Hospital
University of British Columbia
Vancouver, British Columbia
Canada V6Z1Y6

Dr. Philip A. Bromberg
Department of Medicine
School of Medicine
University of North Carolina
Chapel Hill, NC 27514

Dr. George L. Carlo
Dow Chemical, U.S.A.
1803 Building, U.S. Medical
Midland, MI 48640

Dr. Larry D. Claxton
Health Effects Research Laboratory
MD-68
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Donald L. Dungworth
Department of Veterinary Pathology
School of Veterinary Medicine
University of California
Davis, CA 95616

Dr. Richard Ehrlich
Life Sciences Division
Illinois Institute of Technology
Research Institute
Chicago, IL 60616

Dr. Robert Frank
Department of Environmental
Health Sciences
Johns Hopkins School of Hygiene
and Public Health
615 N. Wolfe Street
Baltimore, MD 21205

Dr. Milan J. Hazucha
School of Medicine
Center for Environmental Health
and Medical Sciences
University of North Carolina
Chapel Hill, NC 27514

Dr. Donald H. Horstman
Health Effects Research Laboratory
MD-58
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Steven M. Horvath
Institute of Environmental Stress
University of California
Santa Barbara, CA 93106

Dr. George J. Jakab
Department of Environmental
Health Sciences
Johns Hopkins School of Hygiene
and Public Health
615 N. Wolfe St.
Baltimore, MD 21205

Dr. Robert J. Kavlock
Health Effects Research Laboratory
MD-67
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Reviewers (cont'd)

Dr. Thomas J. Kulle
Department of Medicine
School of Medicine
University of Maryland
Baltimore, MD 21201

Dr. Michael D. Lebowitz
Department of Internal Medicine
College of Medicine
University of Arizona
Tucson, AZ 85724

Dr. Robert C. MacPhail
Health Effects Research Laboratory
MD-74B
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. William F. McDonnell
Health Effects Research Laboratory
MD-58
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Myron A. Mehlman
Environmental Affairs and
Toxicology Department
Mobil Oil Corporation
P.O. Box 1026
Princeton, NJ 08540

Dr. Harold A. Menkes
Department of Environmental
Health Sciences
Johns Hopkins School of Hygiene
and Public Health
615 N. Wolfe Street
Baltimore, MD 21205

Dr. Phyllis J. Mullenix
Forsyth Dental Center
140 The Fenway
Boston, MA 02115

Dr. Mohammad G. Mustafa
Division of Environmental and
Nutritional Sciences
School of Public Health
University of California
Los Angeles, CA 90024

Dr. Russell P. Sherwin
Department of Pathology
University of Southern California
Los Angeles, CA 90033

Dr. Robert J. Stephens
Division of Life Sciences
SRI International
333 Ravenwood Avenue
Menlo Park, CA 94025

Dr. David L. Swift
Department of Environmental
Health Sciences
Johns Hopkins School of Hygiene
and Public Health
615 N. Wolfe Street
Baltimore, MD 21205

Ms. Beverly E. Tilton
Environmental Criteria and Assessment
Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Jaroslav J. Vostal
Executive Department
General Motors Research Laboratories
Warren, MI 48090

SCIENCE ADVISORY BOARD
CLEAN AIR SCIENTIFIC ADVISORY COMMITTEE

The substance of this document was reviewed by the Clean Air Scientific Advisory Committee of the Science Advisory Board in public sessions.

SUBCOMMITTEE ON OZONE

Chairman

Dr. Morton Lippmann
Professor
Department of Environmental Medicine
New York University Medical Center
Tuxedo, New York 10987

Members

Dr. Mary O. Amdur
Senior Research Scientist
Energy Laboratory
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Dr. Eileen G. Brennan
Professor
Department of Plant Pathology
Martin Hall, Room 213, Lipman Drive
Cook College-NJAES
Rutgers University
New Brunswick, New Jersey 08903

Dr. Edward D. Crandall
Professor of Medicine
School of Medicine
Cornell University
New York, New York 10021

Dr. James D. Crapo
Associate Professor of Medicine
Chief, Division of Allergy, Critical Care and Respiratory Medicine
Duke University Medical Center
Durham, North Carolina 27710

Dr. Robert Frank
Professor of Environmental Health Sciences
Johns Hopkins School of Hygiene and Public Health
615 N. Wolfe Street
Baltimore, Maryland 21205

Professor A. Myrick Freeman II
Department of Economics
Bowdoin College
Brunswick, Maine 04011

Dr. Ronald J. Hall
Senior Research Scientist and Leader
Aquatic and Terrestrial Ecosystems Section
Ontario Ministry of the Environment
Dorset Research Center
Dorset, Ontario POA1EO

Dr. Jay S. Jacobson
Plant Physiologist
Boyce Thompson Institute
Tower Road
Ithaca, New York 14853

Dr. Warren B. Johnson
Director, Atmospheric Science Center
SRI International
333 Ravenswood Avenue
Menlo Park, California 94025

Dr. Jane Q. Koenig
Research Associate Professor
Department of Environmental Health
University of Washington
Seattle, Washington 98195

Dr. Paul Kotin
Adjunct Professor of Pathology
University of Colorado Medical School
4505 S. Yosemite, #339
Denver, Colorado 80237

Dr. Timothy Larson
Associate Professor
Environmental Engineering and Science Program
Department of Civil Engineering
University of Washington
Seattle, Washington 98195

Professor M. Granger Morgan
Head, Department of Engineering and Public Policy
Carnegie-Mellon University
Pittsburgh, Pennsylvania 15253

Dr. D. Warner North
Principal
Decision Focus Inc., Los Altos Office Center, Suite 200
4984 El Camino Real
Los Altos, California 94022

Dr. Robert D. Rowe
Vice President, Environmental and Resource Economics
Energy and Resources Consultants, Inc.
207 Canyon Boulevard
Boulder, Colorado 80302

Dr. George Taylor
Environmental Sciences Division
P.O. Box X
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

Dr. Michael Treshow
Professor
Department of Biology
University of Utah
Salt Lake City, Utah 84112

Dr. Mark J. Utell
Co-Director, Pulmonary Disease Unit
Associate Professor of Medicine and Toxicology in Radiation Biology and Biophysics
University of Rochester Medical Center
Rochester, New York 14642

Dr. James H. Ware
Associate Professor
Harvard School of Public Health
Department of Biostatistics
677 Huntington Avenue
Boston Massachusetts 02115

Dr. Jerry Wesolowski
Air and Industrial Hygiene Laboratory
California Department of Health
2151 Berkeley Way
Berkeley, California 94704

Dr. James L. Whittenberger
Director, University of California Southern Occupational Health Center
Professor and Chair, Department of Community and Environmental Medicine
California College of Medicine
University of California - Irvine
19772 MacArthur Boulevard
Irvine, California 92717

Dr. George T. Wolff
Senior Staff Research Scientist
General Motors Research Labs
Environmental Science Department
Warren, Michigan 48090

PROJECT TEAM FOR DEVELOPMENT OF Air Quality Criteria for Ozone and Other Photochemical Oxidants

Ms. Beverly E. Tilton, Project Manager
and Coordinator for Chapters 1 through 5, Volumes I and II
Environmental Criteria and Assessment Office (MD-52)
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Mr. Norman E. Childs
Environmental Criteria and Assessment Office (MD-52)
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. J.H.B. Garner
Coordinator for Chapters 7 and 8, Volume III
Environmental Criteria and Assessment Office (MD-52)
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Mr. Thomas B. McMullen
Environmental Criteria and Assessment Office (MD-52)
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Mr. James A. Raub
Coordinator for Chapters 10 through 13, Volumes IV and V
Environmental Criteria and Assessment Office (MD-52)
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. David T. Tingey
Coordinator for Chapter 7, Volume III
Environmental Research Laboratory
U.S. Environmental Protection Agency
200 S.W. 35th Street
Corvallis, OR 97330

9. TOXICOLOGICAL EFFECTS OF OZONE AND OTHER PHOTOCHEMICAL OXIDANTS

9.1 INTRODUCTION

This chapter discusses the effects of ozone on experimental animals. Carefully controlled studies of the effects of ozone on animals are particularly important in elucidating subtle effects not easily found in man through epidemiological studies and in identifying chronic toxicity not apparent from short-term controlled human exposures. Animal studies allow investigations into the effects of ozone exposure over a lifetime, uncomplicated by the presence of other pollutants. In the animal experiments presented here, a broad range of ozone concentrations has been studied but emphasis has been placed on recent studies at 1960 $\mu g/m^3$ (1 ppm) of ozone or less. Higher concentrations have been cited when the data add to an understanding of mechanisms. Concentrations of 1 ppm or greater cannot be studied ethically in man because of the toxicity of even short-term exposures.

A majority of the literature describes the effects of ozone on the respiratory tract, but extrarespiratory system effects have now been noted and are documented in this chapter. Most of the studies utilize invasive methods that require sacrifice of the animals on completion of the experiment; thus, the studies would be impossible to perform in human subjects. Noninvasive methods of examining most of these endpoints are not readily available.

Emphasis has been placed on the more recent literature published after the prior criteria document (U.S. Environmental Protection Agency, 1978); however, older literature has been reviewed again in this chapter. As more information on the toxicity of ozone becomes available, a better understanding of earlier studies is possible and a more detailed and comprehensive picture of ozone toxicity is emerging. The literature used in developing this chapter is set out in a series of tables. Not all of the literature cited in the tables appears in the detailed discussion of the text, but citations are provided to give the reader more details on the background from which the text is drawn.

In selecting studies for consideration, a detailed review of each paper has been completed. This review included an evaluation of the exposure methods; the analytical method used to determine the chamber ozone concentration; the calibration of the ozone monitoring equipment and the analytical methods used (wherever possible); the species, strain, age and physical characteristics of the animals; the technique used for obtaining samples; and the appropriateness of the technique used to measure the effect. In interpreting the results, the number of animals used, the appropriateness and results of the statistical analysis, the degree to which the results conform with past studies, and the appropriateness of the interpretation of the results are considered. No additional statistical analysis beyond that reported by the author has been undertaken. Unless otherwise stated, all statements of effects in the text are statistically significant at $p \leq 0.05$. Many reports, especially in the older literature, do not present sufficient information to permit the assessment described above. However, should a particular study not meet all of these criteria, but provide reasonable data for consideration, a disclaimer is provided in the text and/or tables.

In this chapter, a discussion of the regional respiratory dosimetry of ozone in common laboratory animal species is presented and compared to human dosimetry. Morphological alterations of the lungs of animals exposed to O_3 are described, followed by the effects of ozone on the pulmonary function of animals. The biochemical alterations observed in the ozone-exposed animals are then related to morphological changes and to potential mechanisms of toxicity and biochemical defense mechanisms. The influence of dietary factors, such as vitamins E and C, in animals is discussed with consideration of potential roles in humans. It should be stressed, however, that no evidence for complete protection against ozone toxicity has been found for any factor, dietary or therapeutic. The effects of ozone on the defense mechanisms of the lung against respiratory infectious agents are discussed using the infectivity model system and effects on alveolar macrophages as examples of experimental evidence. This section is followed by a discussion of ozone tolerance in animals. Last, the effects of ozone on a number of extrarespiratory organ systems are discussed to provide insight into potential effects of ozone inhalation in the respiratory system beyond those now well documented.

A brief discussion of the available literature on the effects of other oxidants likely to occur in polluted air as a result of photochemical reactions or other sources of pollution is presented. Peroxyacetyl nitrate, hydrogen peroxide, and automobile exhaust are the principal pollutants studied in these experiments. This section is short because of the general lack of information in this area, but its brevity does not necessarily reflect a general lack of importance.

A summary is provided for all of the sections of the chapter to set the tone for a clearer understanding of the effects of ozone on animals. The major emphasis of this chapter is to provide evidence for the toxicity of ozone which can not, ethically or practically, be obtained from the study of human subjects. The overall health effects of ozone can be judged from three types of studies: animal exposures, controlled human exposures, and epidemiological studies of adventitious human exposures. No single method alone is adequate for an informed judgment, but together they provide a reasonable estimate of the human health effects of ozone on man.

9.2 REGIONAL DOSIMETRY IN THE RESPIRATORY TRACT

A major goal of environmental toxicological studies on animals is the eventual quantitative extrapolation of results to man. One type of information necessary to obtain this goal is dosimetry, which is the specification of the quantity of inhaled material, in this instance ozone (O_3), absorbed by specific sites in animals or man. This information is needed because the local dose (quantity of O_3 absorbed per unit area), along with cellular sensitivity, determines the type and extent of injury. At this time, only dosimetry is sufficiently advanced for discussion here. Until both elements are advanced, quantitative extrapolation cannot be conducted.

At present, there are two approaches to dosimetry, experimental and deterministic mathematical modeling. Animal experiments have been carried out to obtain direct measurements of O_3 absorption; however, experimentally obtaining local lower respiratory tract (tracheobronchial and pulmonary regions) uptake data is currently extremely difficult. Nevertheless, experimentation is important in assessing concepts and hypotheses, and in validating mathematical models that can be used to predict local doses.

Because the factors affecting the transport and absorption of O_3 are general to all mammals, a model that uses appropriate species and/or disease-specific anatomical and ventilatory parameters can be used to describe O_3 absorption in the species and in different-sized, aged, or diseased members of the same species. Models may also be used to explore processes or factors which cannot be studied experimentally, to identify areas needing additional research, and to test our understanding of O_3 absorption in the respiratory tract.

9.2.1 Absorption in Experimental Animals

There have been very few experiments in which measurements of the regional uptake of O_3 or other reactive gases have been determined. Of the several results published, only one is concerned with the uptake of O_3 in the lower respiratory tract; the others deal with nasopharyngeal uptake.

9.2.1.1 Nasopharyngeal Absorption. Nasopharyngeal removal of O_3 lessens the quantity of O_3 delivered to the lung and must be accounted for when estimating the O_3 dose responsible for observed pulmonary effects. Vaughan et al. (1969) exposed the isolated upper airways of beagle dogs to O_3 at a continuous flow of 3.0 L/min and collected the gas below the larynx in a plastic (mylar) bag. One-hundred percent uptake by the nasopharynx was reported for concentrations of 0.2 to 0.4 ppm. Using a different procedure, Yokoyama and Frank (1972) observed 72 percent uptake at 0.26 to 0.34 ppm (3.5 L/min to 6.5 L/min flow rate). They also replicated the procedure of Vaughan et al. and found that O_3 was absorbed on the mylar bag wall. This may account for the difference between the observations of Yokoyama and Frank (1972) and of Vaughan et al. (1969).

Yokoyama and Frank (1972) also observed a decrease in the percent uptake due to increased flow rate, as well as to increased O_3 concentration. For example, with nose breathing and an O_3 concentration of 0.26 to 0.34 ppm, the uptake decreased from 72 percent to 37 percent for a flow rate increase from 3.5 to 6.5 to 35 to 45 L/min. An increase in concentration from 0.26 to 0.34 to 0.78 to 0.80 ppm decreased nose breathing uptake (3.5 to 6.5 L/min flow rate) from 72 percent to 60 percent. Their data, however, indicate that the tracheal concentration increases with increased nose or mouth concentrations. They also demonstrated that the concentration of O_3 reaching the trachea depends heavily on the route of breathing. Nasal uptake significantly exceeded oral uptake at flow rates of both 3.5 to 6.5 and 35 to 45 L/min. For a given flow rate, nose breathing removed 50 to 68 percent more O_3 than did mouth breathing.

Moorman et al. (1973) compared the loss of O_3 in the nasopharynx of acutely and chronically exposed dogs. Beagles chronically exposed (18 months) to 1 to 3 ppm of O_3 under various daily exposure regimes had significantly higher tracheal concentrations of O_3 than animals tested after 1 day of exposure to corresponding regimes. Moorman et al. (1973) suggested that the differences were due to physiochemical alterations of the mucosal lining in the chronically exposed beagles. When dogs were exposed for 18 months to 1 ppm for 8 hr a day, they had significantly lower tracheal values than those continuously exposed. The average tracheal concentration (0.01 ppm) for the acutely exposed group, however, was not significantly different from that (0.023 ppm) of the 8 hr/day chronic exposure group, when the relative insensitivity of the Mast O_3 meter (unmodified) used to measure the responses is taken into account. Thus, at levels of 1.0 ppm or less, there is no significant evidence that chronic exposure would result in tracheal O_3 concentrations significantly greater than those observed with acute exposure.

Nasopharyngeal removal of O_3 in rabbits and guinea pigs was studied by Miller et al. (1979) over a concentration range of 0.1 to 2.0 ppm. The tracheal O_3 concentration in these two species was markedly similar at a given inhaled concentration and was linearly related to the chamber concentration that was drawn unidirectionally through the isolated upper airways. Ozone removal in the nasopharyngeal region was approximately 50 percent in both species over the concentration range of 0.1 to 2.0 ppm. The positive correlation between the tracheal and chamber concentrations is in agreement with Yokoyama and Frank (1972). Caution needs to be exercised in applying the above results to relate ambient and tracheal concentrations of O_3 since the effects of nasopharyngeal volume and the cyclical nature of breathing are not taken into account. For example, if the tidal volume was less than the nasopharyngeal volume and convection was the only process of axial transport, then no O_3 would be delivered to the tracheal opening, regardless of the percent uptake measured for unidirectional flow.

9.2.1.2 Lower Respiratory Tract Absorption. Morphological studies on animals suggest that O_3 is absorbed along the entire respiratory tract; it penetrates further into the peripheral nonciliated airways as inhaled O_3 concentrations increase (Dungworth et al., 1975b). Lesions were found consistently in the trachea and proximal bronchi and between the junction of the conducting airways and the gaseous exchange area; in both regions, the severity of damage decreases distally. In addition, several studies have reported the most severe or prominent lesions to be in the centriacinar region (see section 9.3).

No experiments determining O_3 tissue dose at the generational or regional level have been reported; however, there is one experiment concerned with the uptake of O_3 by the lower respiratory tract. Removal of O_3 from inspired air by the lower airways was measured by Yokoyama and Frank (1972) in dogs that were mechanically ventilated through a tracheal cannula. In the two ranges of O_3 concentrations studied, 0.7 to 0.85 ppm and 0.2 to 0.4 ppm, the rate of uptake was found to vary between 80 and 87 percent when the tidal volume was kept constant and the respiratory pump was operated at either 20 or 30 cycles/min. This estimate of uptake applies to the lower respiratory tract as a whole; it does not describe uptake of O_3 by individual regions or generations.

9.2.2 Ozone Dosimetry Models

9.2.2.1 Modeling Nasal Uptake. LaBelle et al. (1955) considered the absorption of gases in the nasal passages to be similar to absorption on wetted surfaces of distillation equipment and scrubbing towers and applied the theory and models of these devices to the nasal passages of rats. By associating biological parameters of rats with the chemical engineering device parameters of the model, they calculated the percent of penetration of several gases to the lung. They concluded that Henry's law constant is the major variable in determining penetration. Based on these calculations, The National Academy of Sciences (National Research Council, 1977) concluded that the model predicts 99 percent penetration for O_3. This is much more than that measured by Yokoyama and Frank (1972) or by Miller et al. (1979). Several possible reasons for the differences were discussed (National Research Council, 1977), but the major factor was considered to be that the model does not account for the reactions of O_3 in the mucus and epithelial tissue.

Aharonson et al. (1974) developed a model for use in analyzing data from experiments on the uptake of vapors by the nose. The model was based on the assumptions of quasi-steady-state flow, mass balance, and proportionality of flux of a trace gas at the air-mucus interface to the gas-phase partial pressure of the trace gas and a "local uptake coefficient" (Aharonson et al., 1974). The model was applied to data from their own experiments on the removal of acetone and ether in dog noses. They also applied the model to the O_3 uptake data of Yokoyama and Frank (1972) and concluded that the uptake coefficient (average mass transfer coefficient) for O_3, as well as for the other gases considered, increases with increasing air flow rate.

9.2.2.2 Lower Respiratory Tract Dosimetry Models. There are three models for which published results are available. The model of McJilton et al. (1972) has been discussed and simulation results for O_3 absorption in each generation of the human lower respiratory tract are available (National Research

Council 1977; Morgan and Frank, 1977). Two models have been developed by Miller and co-workers. A detailed description of the formulation of the first and earliest mathematical model of Miller and co-workers is found in Miller (1977) and major features are given in Miller et al. (1978b) and Miller (1979). Results, using this model, of simulations of the lower respiratory tract absorption of O_3 in humans, rabbits, and guinea pigs are in Miller (1977, 1979) and Miller et al. (1978b). The formulation of the second model of Miller and co-workers, as well as results of simulating lower respiratory tract absorption of O_3 in humans, appears in Miller et al. (1985).

Because all of the above models were developed to simulate the local absorption of O_3, they have much in common. This is especially true with respect to the following areas: formulation of O_3 transport in the airspaces or lumen of the airways, use of morphometric models of the lower respiratory tract, and inclusion of a liquid lining that coats the tissue walls of the airspaces or lumen of the airways.

In each model the descriptions of O_3 transport and absorption in the lumen are based on a one-dimensional differential equation relating axial convection, axial dispersion or diffusion, and the loss of O_3 by absorption at the gas-liquid interface. The use of a one-dimensional approximation has been very common in modeling the transport of gases such as O_2, N_2, etc., in the lower respiratory tract (see Scherer et al., 1972; Paiva, 1973; Chang and Farhi, 1973; Yu, 1975; Pack et al., 1977; Bowes et al., 1982). The approximation is appropriate for O_3 as well.

The models of Miller and co-workers took into account effective axial dispersion in the airways by using an effective dispersion coefficient based on the results of Scherer et al. (1975). McJilton's model did not take this factor into account (Morgan and Frank, 1977). However, this may not be an important difference since Miller et al. (1985) report that results are little affected by different values of the coefficient. Also, Pack et al. (1977) and Engel and Macklem (1977) reported results that indicate an insensitivity of airway concentrations to the effective dispersion coefficient.

Airway or morphometric zone models such as those of Weibel (1963) and Kliment (1973) were used to define the lengths, radii, surface areas, cross-sectional areas, and volumes of the airways and air spaces of each generation or zone. The breathing pattern was assumed sinusoidal; however, dimensions were held constant throughout the breathing cycle. The physical properties of the liquid lining were assumed to be those of water. The lining thickness depended on generation or zone, being thicker in the upper airways than in the lower.

The flux of O_3 from the lumen or airspaces to the liquid lining was defined in each model in terms of a mass-transfer coefficient. McJilton et al. (1972) made the assumption that radial mass transfer was controlled by the absorbing medium and estimated the transfer coefficient from empirical data on the physical properties (not chemical) of the medium and of O_3 (National Research Council, 1977). In the first model of Miller and co-workers (Miller, 1977; Miller et al., 1978b) the radial dependence of the luminal O_3 concentration was assumed to vary quadratically with the radius. From this formulation, the gas-phase mass transfer was determined. For their later model, the gas phase mass transfer coefficient was defined in terms of a Sherwood number. In both models the gas phase coefficient was combined with the mass transfer coefficient of the absorbing medium (which depended on the chemical and physical properties of the absorbing medium and of O_3) to obtain the overall transfer coefficient. However, Miller et al. (1985) conclude, based on the data available for the absorbing medium, that radial mass transfer is controlled by the medium, making specification of the gas phase mass transfer coefficient unnecessary.

The main differences in the models are the mechanisms of absorption and their formulation. In the model of McJilton et al. (1972) and in the early model of Miller and co-workers (Miller, 1977; Miller et al., 1978b) there is only one compartment, the liquid lining, which can absorb unreacted O_3. In the later model of Miller et al. (1985) there are three absorbing compartments, liquid lining, tissue, and capillary or blood (in the pulmonary region where the air-blood tissue barrier is very thin). Further, O_3 is known to react chemically with constituents of the absorbing medium(s). This aspect, included by Miller and co-workers, was not included in McJilton's model. The inclusion resulted in significant differences between the tissue dose pattern curves in the tracheobronchial region predicted by the models. In addition, McJilton's model predicts a dose curve (equivalent to a tissue dose curve because of no mucous reactions) in the tracheobronchial region that has its maximum at the trachea and decreases distally to the thirteenth or fourteenth generation (see Figure 7-5 in National Research Council, 1977). By contrast, the models of Miller and co-workers predict the tissue dose to be a minimum at the trachea and to increase distally to the pulmonary region.

The concentration of ozone in tissue and at the liquid-tissue interface was assumed to be zero by McJilton et al. (1972) (National Research Council, 1977; Morgan and Frank, 1977) and in the first model of Miller and co-workers (Miller, 1977; Miller et al., 1978b). The interpretation was that this boundary condition means that O_3 reacts (chemically) instantaneously when it reaches the tissue. Miller et al. (1978b) define the tissue dose as that quantity of O_3 per unit area reacting with or absorbed by the tissue at the liquid-tissue interface.

The first model of Miller and co-workers took into account the reaction of O_3 with the unsaturated fatty acids (UFA) and amino acids in the mucous-serous lining. Reactions of O_3 with other components (such as carbohydrates) were not included in the model because of insufficient information (Miller, 1977; Miller et al., 1978b). The O_3-UFA and O_3-amino acid reactions were assumed fast enough so that an instantaneous reaction scheme based on that outlined in Astarita (1967) could be used. The scheme required the specification of the production rate of the UFA and amino acids in each mucous-lined generation. These rates were estimated by using tracheal mucous flow data, the surface area of the tracheobronchial region, the concentrations of the specific reactants known to react with O_3, and the assumption that the production rate decreased distally (Miller, 1977; Miller et al., 1978b).

Although the instantaneous reaction scheme is a good preliminary approach to treating O_3 reactions in the mucous-serous lining, its use is not completely justifiable. Second-order rate constants of O_3 with some of the UFA present in mucus indicate that although they are large (Razumovskii and Zaikov, 1972), they are less than the diffusion-limited rates necessary for the instantaneous reaction scheme. Experimental evidence (Mudd et al., 1969) suggests that the reactions of O_3 with amino acids are very rapid. Rate constants for these reactions and others are not known; thus, the information available is scanty, which makes the specification of a reaction mechanism or reaction scheme difficult and assumptions necessary.

The approach to chemical reactions used in the later model (Miller et al., 1985) goes a long way in addressing the above criticisms. The reaction of O_3 with biochemical constituents is assumed to be bimolecular; however, the concentration of the constituents is considered to be large enough so as not to be depleted by the reactions. Hence, the model uses a pseudo first order reaction scheme in which the pseudo first order rate constant is the product of the bimolecular rate constant and the concentration of the biochemical constituents that react with O_3.

For modeling purposes, Miller et al. (1985) consider that only the reaction of ozone with the UFA is important, using the O_3-oleic acid rate constant of Razumovskii and Zaikov (1972) for the O_3-UFA reaction. They point out that although O_3 reacts with amino acids and other constituents, rate constants are not known and that Bailey (1978) estimates the reaction of O_3 with UFA to be $\sim 10^3$ times faster than the O_3-amino acid reaction, justifying omitting amino acids from consideration.

9.2.3 Predictions of Lower Respiratory Tract Ozone Dosimetry Modeling

The predictions of lower respiratory tract dosimetry models are reviewed by illustrating the results of simulations, by comparing predictions to experimental observations, and by describing uses for dosimetry models.

The following discussion of modeling results of lower respiratory tract absorption is based mainly on simulations using the first model of Miller and co-workers (Miller, 1977; Miller et al., 1978b). This is because the model includes the important effects of O_3 reactions in the mucous-serous lining and because simulations of O_3 absorption in laboratory animals are available.

Simulations of O_3 absorption in different animals can be carried out by modifying input parameters of the computer program that solves the mathematical equations. These input parameters, which characterize an animal, include the number and dimensions of the airways, tidal volume, length of time of one breath, etc. The airway and alveolar dimensions of Weibel (1963) were used for the simulation of O_3 uptake in humans. For the rabbit and guinea pig, Miller and co-workers used the morphometric zone models of Kliment (1973). The zone model is a less detailed model than the generationally based airway model of Weibel (1963) since more than one generation corresponds to a zone in an animal; they were used because they were the only complete (tracheobronchial and pulmonary regions) "airway" models available at the time. However, Schreider and Hutchens (1980) criticize the guinea pig model of Kliment (1972, 1973) as having a lung volume that is too low, suggesting the possibility of incomplete casts. Since the same method also was used by Kliment (1972, 1973) for the rabbit, this criticism may also apply to this model.

To illustrate simulation results, two aspects of the simulations by Miller and co-workers are considered: (1) the effect of various tracheal concentrations on the tissue dose pattern (tissue dose as a function of zone or generation) in guinea pigs and rabbits; and (2) the similarity between the dose patterns of guinea pigs, rabbits, and humans.

9.2.3.1 Illustration of Dosimetry Simulations. Figure 9-1 is a set of plots of the tissue dose for one breath versus zone for various tracheal O_3 concentrations for the rabbit and the guinea pig. All curves have the same general characteristics. Independent of the inhaled concentration, the model predicts that the first surfactant-lined zone (first non-mucous-lined or first zone in the pulmonary region), zone 6, receives the maximal dose of O_3. Although the model predicts uptake of O_3 by respiratory tissue (zones 6, 7, and 8) for all tracheal concentrations studied (62.5 to 4000 $\mu g/m^3$), the penetration of O_3 to the tissue in the airways lined by mucus depended on the tracheal concentration and the specific animal species. For example, as illustrated in Figure 9-1 for the tracheal O_3 concentration of 1000 $\mu g/m^3$, no O_3 reaches the airway tissue of the rabbit until zone 3, whereas O_3 is predicted to penetrate to the tissue in guinea pig airways in all zones. However, at the two lowest tracheal concentrations plotted, 250 and 62.5 $\mu g/m^3$, no penetration occurs until zones 4 and 6, respectively, for both animals. The dependence of penetration on tracheal O_3 concentration is a result of the instantaneous reaction scheme used to describe chemical reactions. Penetration does occur in the simulation of uptake in humans using the newer model of Miller and co-workers (1985), as depicted in Figure 9-2, and can be expected to occur in future animal simulations.

The similarity of the predicted dose patterns in rabbits and guinea pigs extends to the simulation of O_3 uptake in humans. Figure 9-2 compares the tissue dose for the three species for a tracheal concentration of 500 $\mu g/m^3$ (0.26 ppm) using results of the first model for the three species and the newer model results for man. In Figure 9-2, the guinea pig and rabbit tissue doses are plotted in the form of a histogram to allow a comparison to human dosimetry data that are expressed as a function of airway generation.

For the earlier model, the dose patterns of the three species peak at the first surfactant-lined zone (6) or in generation 17 (which is in zone 6). Also, O_3 penetrates to the tissue everywhere in the pulmonary region (zones > 5 and generations > 16); however, O_3 is not predicted to penetrate to the tissue before zone 3 for the rabbit and guinea pig or before zone 5 (generations 12-16) for man.

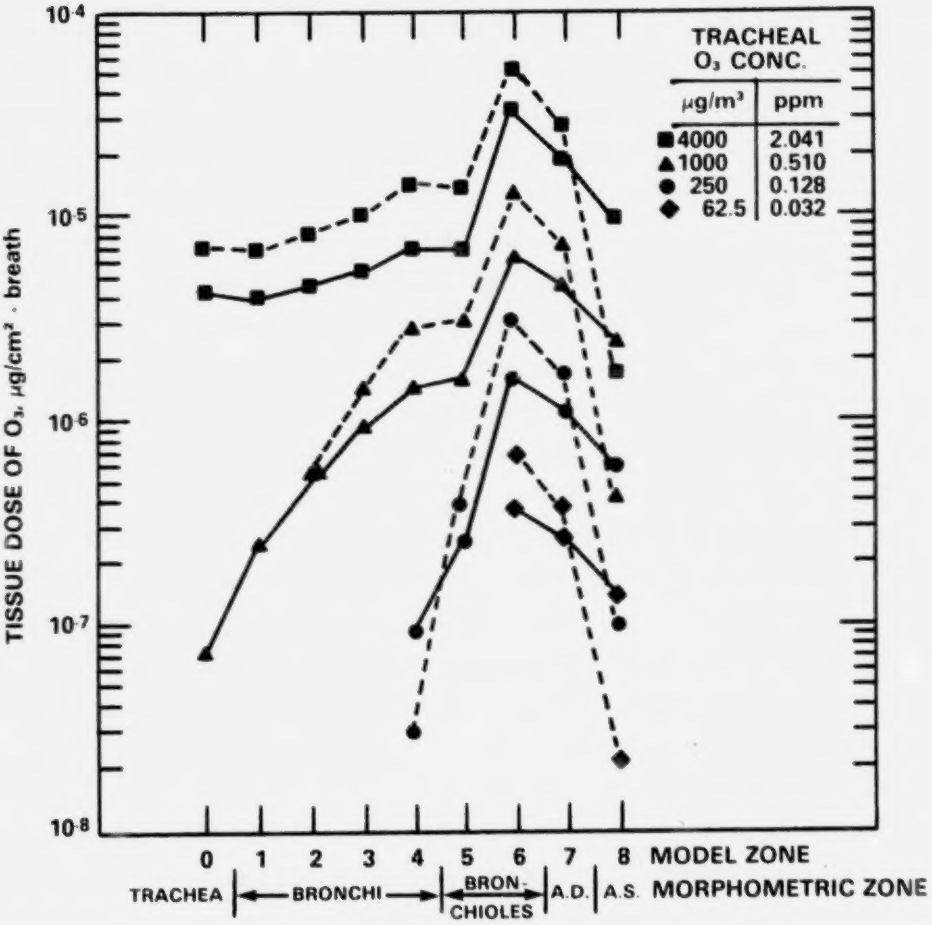

Figure 9-1. Predicted tissue dose for several tracheal O_3 concentrations for rabbit (– – –) and guinea pig (——). See text for details. (A.D. = alveolar duct; A.S. = alveolar sac).

Source: Adapted from Miller et al. (1978b).

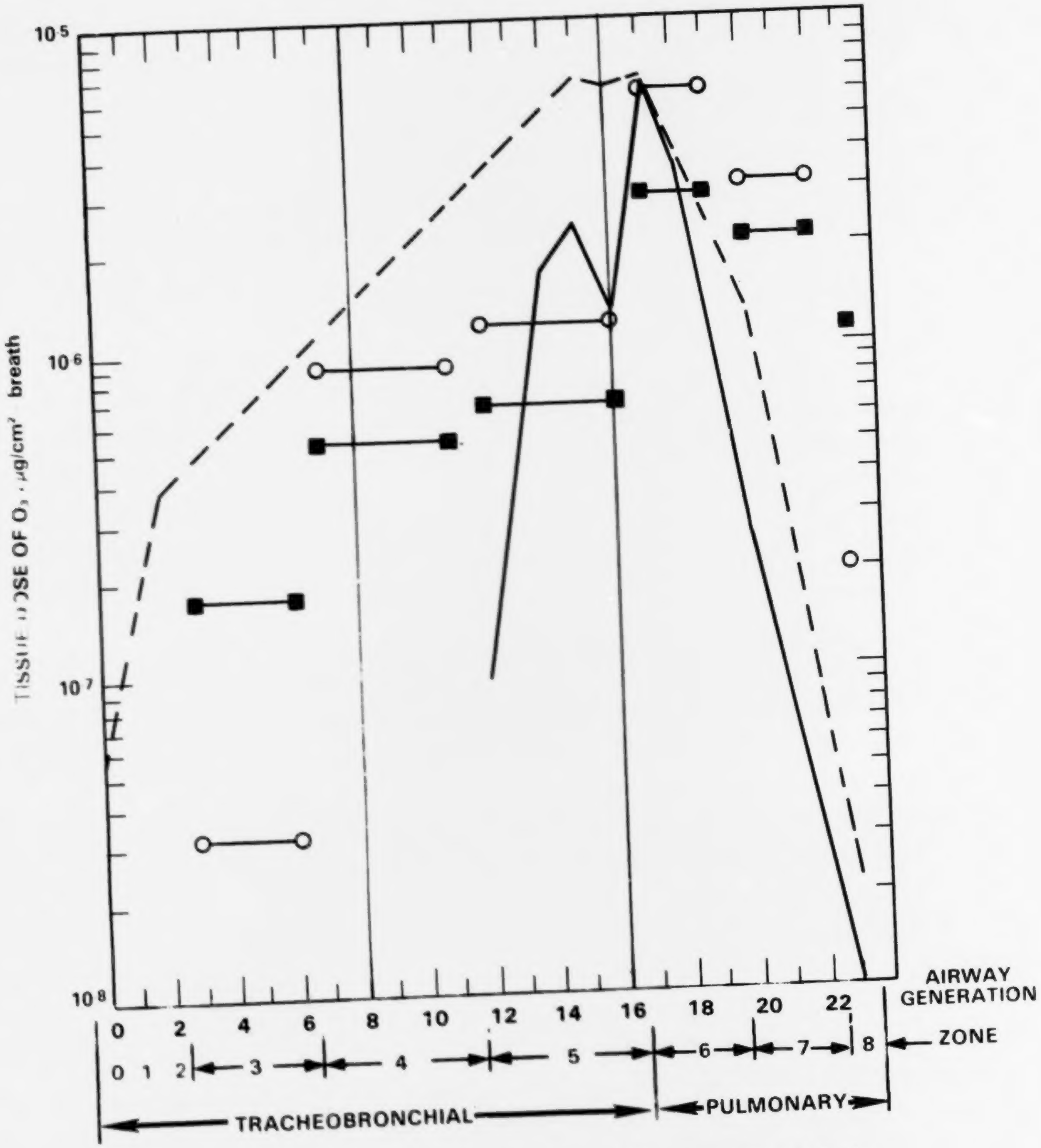

Figure 9-2. Tissue dose versus zone for rabbit (○) and guinea pig (■); and tissue dose versus airway generation for human (——, earlier model; – – –, newer model). Tracheal O_3 concentration is 500 $\mu g/m^3$ (0.26 ppm). See text for details.

Source: Guinea pig, rabbit and earlier human simulations adapted from Miller et al. (1978b); newer model results for human adapted from Miller et al. (1985).

All of the earlier simulations presented by Miller and co-workers (Miller, 1977; Miller et al., 1978b; Miller, 1979) share the following characteristics: (1) the maximal tissue dose occurs in the first surfactant-lined zone or generation; (2) O_3 penetrates to tissue everywhere in the pulmonary region (zones >5), decreasing distally from the maximum; and (3) the onset of O_3 penetration to mucous-lined tissue, as well as dose in general, depends on tracheal O_3 concentration, animal species, and the breathing pattern. These general characteristics of tissue dose pattern are independent of the two airway models used.

Results using the newer model to simulate tissue dose in humans are also illustrated in Figure 9-2. Comparison of dose values for the two human simulations show the most notable differences in the conducting airways (generations 0 to 16). The new and old results are similar in that both models predict relatively low doses in the upper airways, a maximum in the first pulmonary generation, and then a rapid decline in dose distally. There is no reason to assume that these features would be missing in simulation results for laboratory animals using the newer model.

9.2.3.2 Comparison of Simulations to Experimental Data. There are no quantitative experimental observations with which to compare the results of modeling the local uptake of O_3 in the lower respiratory tract. Yokoyama and Frank (1972) observed 80 to 87 percent uptake of O_3 by the isolated lower respiratory tract of dogs. With the earlier model, Miller et al. (1978b) predict a 47 percent uptake for humans; the newer model predicts 89 percent (Miller et al., 1985). Because of differences between the two species, comparing the experimental and simulated results is most likely inappropriate.

Morphological studies in animals report damage throughout the lower respiratory tract. Major damage, and in some cases the most severe damage, is observed to occur at the junction between the conducting airways and the gas exchange region, and to decrease distally (see Section 9.3.1.1). For the animals simulated by Miller and co-workers, the maximal tissue dose of O_3 is predicted to occur at this junction, with the dose curve decreasing rapidly for more distal regions. Thus, in the pulmonary region, the model results are in qualitative agreement with experimental observations.

Damage is also observed in the trachea and bronchi of animals (see Sections 9.3.1.1.1.2 and 9.3.1.1.1.3). In the animals modeled, the early model of Miller and co-workers either predicts significantly less tissue dose in the upper airways compared to the dose in the first zone of the pulmonary region or it predicts no penetration to the tissue in the upper portion of the conducting airways (see Figure 9-2). Based on simulation results using the newer model for humans (Miller et al., 1985), one can infer that this model will predict non-zero tissue doses in the upper airways of animals, but that these doses also will be significantly less than predicted for the centriacinar airways. The observations of damaged upper and lower respiratory tract airways in the same animal and the predictions of significantly different tissue doses in the two regions appear inconsistent. However, much of the reported damage in the trachea and bronchi is associated with the cilia of ciliated cells, which in current model formulations are not part of the tissue. The cilia extend into the hypophase (perciliary) portion of the mucous-serous layer, and the dosimetry models do not distinguish the cilia of the ciliated cells as a separate component of this layer. Thus, relatively low predicted tissue dose should not be interpreted as predicting no damage to cilia. Likewise, the frequent reporting of cilia being damaged following O_3 exposure should not be interpreted necessarily as an indication of O_3 tissue dose since the model definition of tissue does not currently include cilia. The inclusion of a "cilia compartment" in future dosimetry models may be helpful. There are also other factors that complicate our understanding of ozone toxicity, such as the possibility of O_3 reaction products being toxic or differences in cell sensitivity that may prevent explanations of observed effects based on dosimetry modeling alone.

9.2.3.3 Uses of Predicted Dose. Model-predicted doses can be used to estimate comparable exposure levels that produce the same dose in different species or different members of the same species for use in comparing toxicological data. One can simulate tissue dose for several species for the same time for a range of tracheal O_3 concentrations. The doses for a specific zone or generation, for each species, can be plotted versus tracheal or ambient O_3 concentration. By using such plots and information on nasopharyngeal removal, the ambient concentration necessary to produce the same dose in different species can be estimated. Also, the relative quantity of O_3 delivered to a zone or generation in a given species for the same time span and ambient concentration can be predicted from the same graph. If the same biological parameters have not been measured in these species at dose-equivalent exposure levels, the procedure can be used to scale data and to design new studies to fill gaps in the current data base.

To illustrate the above procedure, Miller (1979) calculated exposure levels of O_3, giving the same respiratory bronchiolar dose in rabbits, guinea pigs, and man. Considering the discussion on nasopharyngeal removal (Section 9.2.1.1) and the question concerning the guinea pig and rabbit anatomical models (Section 9.2.3), these calculations are mainly useful for illustrative purposes.

9.3 EFFECTS OF OZONE ON THE RESPIRATORY TRACT

9.3.1 Morphological Effects

The many similarities and differences in the structure of the lungs of man and experimental animals were the subject of a recent workshop entitled "Comparative Biology of the Lung: Morphology", which was sponsored by the Lung Division of the National Institute of Blood, Heart, and Lung Diseases (National Institutes of Health, 1983). These anatomical differences complicate but do not necessarily prevent qualitative extrapolation of risk to man. Moreover, because the lesions due to O_3 exposure are similar in many of the species studied (see Table 9-1), it appears likely that many of the postexposure biological processes of animals could also occur in man.

9.3.1.1 Sites Affected. The pattern and distribution of morphological lesions are similar in the species studied. Their precise characteristics depend on the location (distribution) of sensitive cells and on the type of junction between the conducting airways and the gaseous exchange area.

The upper or extrathoracic airways consist of the nasal cavity, pharynx, larynx, and cervical trachea. Except for a few sites, the lining epithelium is ciliated, pseudostratified columnar, with mucous (goblet) cells; it rests on a lamina propria or submucosa that contains numerous mucous, serous, or mixed glands and vascular plexi. Sites with differing structure include the vestibule of the nasal cavity and portions of the pharynx and larynx, which tend to have stratified squamous epithelium, and those portions of the nasal cavity lined by olfactory epithelium, which contain special bipolar neurons and glands associated with the sense of smell. Significant morphological differences exist among the various animal species used for O_3 exposures as well as between most of them and man (Schreider and Raabe, 1981; Gross et al., 1982). With the exception of the cervical trachea, these structures have received little attention with respect to O_3 sensitivity, but O_3 removal through "scrubbing" has been studied (Yokoyama and Frank, 1972; and Miller et al., 1979).

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE

Ozone concentration μg/m³	ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
196	0.1	UV, NBKI	7 days, continuous	Two of six fed with "basal" vitamin E diet had increased centriacinar AMs (SEM, LM).	Rat	Plopper et al., 1979
392	0.2			Centriacinar accumulation of AMs, commonly in clumps of 3-5. Occasionally cilia were reduced in number, nonciliated cells, some reduction in height.		
196	0.1	UV, NBKI	7 days, continuous	Five of six fed E-deficient "basal" diet had centriacinar AMs and bronchiolar epithelial lesions (SEM). Four of six fed "basal" diet +11 ppm E had lesser but similar lesions. One of six fed "basal" diet +110 ppm E had lesser lesions.	Rat	Chow et al., 1981
392	0.2	MAST, NBKI	30 days, continuous	Increased lung volume, mean chord length, and alveolar surface area. Lung weight and alveolar number did not change. Decrease in lung tissue elasticity. Parenchyma appeared "normal" by LM.	Rat	Bartlett et al., 1974
392 686 980 1568	0.2 0.35 0.5 0.8	UV, NBKI	7 days, 8 hr/day	Respiratory bronchiolitis at all concentrations. Increased AMs. Bronchiolar epithelium both hyperplastic and hypertrophic. Increased alveolar type 2 cells. Random foci of short, blunt cilia or absence of cilia (LM, SEM, TEM).	Monkey (Rhesus and Bonnet)	Dungworth et al., 1975b
392 686	0.2 0.35	UV, NBKI	7 days, 8 hr/day	All exposed monkeys had LM & EM lesions. Trachea and bronchi had areas of shortened or less dense cilia. RBs had AM accumulation and cuboidal cell hyperplasia. Alveoli off RBs had AM accumulations and increased type 2 cells. RB walls of the 0.35-ppm group were often thickened due to mild edema and cellular infiltration.	Monkey (Bonnet)	Castleman et al., 1977
392 980 1568	0.2 0.5 0.8	UV, NBKI	7 days, 8 hr/day or 24 hr/day	Exposed groups gained less weight. Focal areas of missing or damaged cilia in trachea and bronchi. TB nonciliated (Clara) cells were shorter and had increased surface granularity and less smooth endoplasmic reticulum. Ciliated cells of TB had fewer cilia and focal blebs. Centriacinus had clusters of AMs and PMNs. Type 1 cells swollen and fragmented and type 2 cells frequently in pairs or clusters. Proximal IAS were minimally thickened. Lesions in 0.2 rats were mild (LM, SEM, TEM). Only slight differences between rats exposed continuously 24 hr/day compared to those exposed only 8 hr/day.	Rat	Schwartz et al., 1976

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
μg/m³	ppm					
392 980 1568	0.2 0.5 0.8	UV, NBKI	20, 50, or 90 days; 8 hr/day	Epithelial changes and PAM accumulations at 90 days were similar to 7-day exposures, but less severe. 0.5- and 0.8-ppm groups had increased centriacinar PAMs at all times. 0.2 ppm and controls could not be separated by "blind" LM examination, nor were there distinguishing EM changes. 90-day 0.8-ppm group had changed the terminal bronchiole/alveolar duct junction to terminal bronchiole/respiratory bronchiole/alveolar duct junctions. TBs had loss or shortened cilia. Nonciliated cells were flattened lumenal surfaces that occasionally occurred in clusters. Proximal alveoli of 20- and 90-day 0.8-ppm groups had thicker blood/air barriers.	Rat	Boorman et al., 1980
392 980 1960	0.2 0.5 1.0	ND, NBKI	4 days, 3 hr/day, exercised in a rotating case alternate 15 min	Exercised control mice have significantly smaller body weights. Both unexercised and exercised mice exposed to 0.5 or 1.0 ppm had smaller body weights and larger lung weight. Exercised mice exposed to 0.2 ppm also had larger lung weights. Other pathology not studied.	Mouse (male, 5 weeks old, ICR-JCL)	Fukase et al., 1978
392	0.2	CHEM	7, 14, 30, 60, 90 days; continuous	Short-term exposures produced a slight degree of tonsil epithelial detachment. Cell infiltration below the epithelium was slight. Long-term exposures caused slight edema of the lacunar epithelium which was destroyed or detached in places. Lymphocyte infiltration also occurred.	Rabbit	Ikematsu, 1978
1960	1.0		10 days, continuous	Tonsil epithelium had a high degree of detachment. Cell saturation occurred below the epithelium. Some protrusion of the tonsil into the oral cavity.		
9800	5.0		3 hr	Strong detachment of the tonsil epithelium. High degree of cell saturation below the epithelium, including lymphocyte infiltration around the blood vessels and swelling of the endothelial cells. Large amount of lymphocytes, viscous liquid, and detached epithelial cells in the tonsilar cavity.		

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
490	0.25	CHEM	6 weeks, 12 hr/day	Centriacinar or proximal alveoli had thicker interalveolar septa with significant increases in epithelium, cellular interstitium, and endothelium. Type 1 and 2 alveolar epithelial cells and macrophages were increased in numbers. Type 1 cells had smaller volumes and surface areas and were thicker.	Rat	Barry et al., 1983; Crapo et al., 1984
510 980 1960	0.26 0.50 1.0	MAST, NBKI	4.7-6.6 hr, endotracheal tube	Desquamation of ciliated epithelium. Focal swelling or sloughing of type 1 cells.	Cat	Boatman et al., 1974
588	0.3	NBKI	16 days, 3 hr/day	SEM, but not LM, showed swollen cilia with hemispheric extrusions and surface roughness. Some adhesion of severely injured cilia occurred. Small, round bodies were frequently noted, mainly in the large airways and proximal bronchioles. Luminal surfaces of the epithelium were often covered with a pseudomembrane. The surfaces of Clara cells showed swellings and round bodies. The surfaces of alveolar ducts and walls showed scattered areas of cytoplasmic swelling and attachment of round bodies. All responses were pronounced in vitamin E-deficient rats. Some rats had chronic respiratory disease.	Rat	Sato et al., 1976a
588	0.3	NBKI	28 weeks, 5 days/week, 3 hr/day	No morphological differences noted between vitamin E-deficient and vitamin E-supplemented groups with the use of SEM and TEM. Exposed and control rats had chronic respiratory disease.	Rat	Sato et al., 1980
588	0.3	UV	6 weeks, 5 days/week, 7 hr/day	Increased LDH positive cells stated to be type 2 cells.	Mouse	Sherwin et al., 1983
588	0.3	ND	1, 5, 11, and 16 days, 3 hr/day	Rats were fed a basal diet with or without vitamin E supplement. Volume density of lamellar bodies in type 2 alveolar epithelial cells were increased. Giant lamellar bodies were seen after 11 days exposure.	Rat	Shimura et al., 1984
686 980 1372 1470 1960	0.35 0.50 0.70 0.75 1.00	ND	1, 2, 4, 5, 6 or 8 days, continuous	Dividing cells were labeled with tritiated thymidine and studied with autoradiographic techniques by using LM. All labeled cells increased and then decreased to near control levels within 4 days. Type 2 cells showed largest change in labeling index.	Rat	Evans et al., 1976b
686 980	0.35 0.50		4 days, continuous, followed by 0.50, 0.70, 0.75 or 1.00 for 1-4 days	Type 2 cells from groups showing adaptation to O_3 were exposed to higher concentrations. Groups exposed to low initial concentration of O_3 (0.35 ppm) did not maintain tolerance. Groups exposed to higher initial concentration (0.50 ppm) demonstrated tolerance.		

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
μg/m³	ppm					
784	0.4	MAST	10 months, 5 days/week, 6 hr/day	All (exposed and control) lungs showed some degree of inflammatory infiltrate possibly due to intercurrent disease. A "moderate" degree of "emphysema" was present in 5 of the 6 exposed rabbits. Lungs of the 6th were so congested that visualization of the mural framework of the alveoli was difficult. Small pulmonary arteries had thickened tunica medias, sometimes due to edema, other times to muscular hyperplasia.	Rabbit (New Zealand)	P'an et al., 1972
784	0.4	NBKI	7 hr/day, 5 days/week, 6 weeks	Lung growth which follows pneumonectomy also occurred following both pneumonectomy and O_3 exposure.	Rabbit	Boatman et al., 1983
980	0.5	ND	2 to 6 hr	Centriacinar type 1 cells were swollen then sloughed. Type 2 cells were not damaged and spread over the denuded basement membrane. In some areas of severe type 1 cell damage, endothelial swelling occurred. Damaged decreased rapidly with distance from TB. Damage was most severe only in the most central 2-3 alveoli. Interstitial edema occasionally observed.	Rat (young males)	Stephens et al., 1974b
980 or 1568	0.5 or 0.8	UV, NBKI	7 continuous days; 2, 4, 6, 8, or 24 hr/day	Centriacinar inflammatory cells (mostly AMs) were counted in SEMs. Dose-related increase in inflammatory cell numbers except in the continuously (24-hr/day) 0.8-ppm exposed rats. Rats exposed 0.5 and 0.8 ppm 24 hr/day had the same intensity of effect.	Rat	Brummer et al., 1977
980	0.5	UV, NBKI	7, 21, and 35 days, continuous	Most severe damage at terminal bronchiole/alveolar duct junction. TB had focal hyperplastic nodules of nonciliated cells. Proximal alveoli had accumulations of macrophages and thickening of IAS by mononuclear cells at 7 days. At 35 days, changes much less evident, but increased type 2 cells.	Mouse (Swiss-Webster; 60 days old; 35-40 g)	Zitnik et al., 1978
980	0.5	ND	2 days	Tolerance was induced by exposure to 0.5 ppm O_3 for 2 days. Challenge was by exposure to 6.0 ppm O_3 for 24 hours. Tolerance was present at 3 days and declined at 7 and 15 days after the initial exposure. When the animals were tolerant, the type 1 alveolar epithelium was thicker, had a smaller surface area and a smaller surface-to-volume ratio.	Rat	Evans et al., 1985
980 1568	0.5 0.8	UV, NBKI	7, 28, or 90 days, continuous, 8 hr/day	Principal lesion was a "low-grade respiratory bronchiolitis" characterized by "intraluminal accumulations of macrophages and hypertrophy and hyperplasia of cuboidal bronchiolar epithelial cells." Conducting airway lesions not apparent by LM, but parallel linear arrays of uniform shortening and reduction of density of cilia by SEM. Kulschitzky-type cells appeared more numerous in exposed.	Monkey (Bonnet)	Eustis et al., 1981

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement^{a,b} method	Exposure duration and protocol	Observed effect(s)^{c}	Species	Reference
980 1568	0.5 0.8	UV, NBKI	7 days, 8 hr/day	All exposed monkeys had lesions. Lesions similar in 0.5- and 0.8-, less severe in 0.5-ppm exposure groups. Patchy areas of epithelium devoid of cilia in trachea and bronchi. Luminal surfaces of RB and proximal alveoli coated with macrophages, a few neutrophils and eosinophils and debris. Nonciliated cuboidal bronchiolar cells were larger, more numerous, and sometimes stratified. Proximal alveolar epithelium thickened by increased numbers of type 2 cells. Progressive decrease in intensity of lesions from proximal to distal orders of RBs.	Monkey (Rhesus, adult)	Mellick et al., 1975, 1977
980	0.5	MAST	60 days 6 hr/day	Both immersion and infusion fixed lungs were studied by LM. Immersion fixed large and middle size bronchi had deeper than normal infolding of the mucosa with increased secretions. The low concentration rats had less severe mucosal infolding, but a greater accumulation of secretions.	Rats	Yokoyama et al., 1984
1960	1.0	NBKI	30 days 3 hr/day			
980 to 3920	0.5 to 2.0	UV, NBKI	7 days, 24 hr/day	Elevated collagen synthesis rates and histologically discernible fibrosis was present at all levels of O_3. 0.5 ppm Minimal or no thickening of walls or evidence of fibrosis. Increased number of cuboidal cells and macrophages present. 0.8-2.0 ppm: Moderate thickening of AD walls and associated IAS by fibroblasts, reticulin and collagen with narrowing of the ducts and alveoli. Thickening decreased with increased length of exposure.	Rat	Last et al., 1979
	0.5 to 1.5		14 days and 21 days, 24 hr/day	0.5 ppm Sometimes minimal thickening of alveolar duct walls with mildly increased reticulin and collagen.		
980	0.5	CHEM, NBKI	6 months, 5 days/week, 6 hr/day	Only O_3 caused pulmonary lesions. Only LM histopathology, no SEM nor TEM. Rats did not have exposure-related pulmonary lesions, except 2 of 70 rats in the O_3 group, which had type 2 hyperplasia and focal alveolitis; 2 of 70 rats from the O_3 + H_2SO_4 group had slight hypertrophy and hyperplasia of bronchiolar epithelium. Guinea pigs exposed to O_3 or O_3 + H_2SO_4 had lesions "near" the TB. Epithelium was hypertrophied and hyperplastic. Macrophages were in centriacinar alveoli. Occasionally proliferation of type 2 cells. Trachea and bronchi had slight loss of cilia, reduction of goblet cells, and mild basal cell hyperplasia. Ozone alone had no effect on body weight gain; lung/body weight ratio; RBCs, hemoglobin, or hematocrit.	Rat and Guinea pig	Cavender et al., 1978
	0.5 O_3 + 10 mg/m³	H_2SO_4				

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
$\mu g/m^3$	ppm					
980	0.5 $0.5\ O_3$ + $1\ mg/m^3$	UV, NBKI H_2SO_4	3, 50, 90, or 180 days; continuous, plus 62 days postexposure, 24 hr/day	H_2SO_4 did not potentiate effects of O_3 alone. Fixed lung volumes were increased at 180 days, but decreased at 62 days postexposure. After 50, 90, 94 180 days all O_3 exposure rats had "bronchiolization of alveoli" or formation of an RB between the TB and ADs. Centriacinar inflammatory cells were significantly increased at all exposure times and after 62 days postexposure. TB lesions were qualitatively similar at 3, 50, 90, and 180 days. Cilia were irregular in number and length. Nonciliated secretory (Clara) cells had flattened apical protrusions and a blebbed granular surface. At 90, but not 180 days, small clusters of nonciliated cells were present in the TB. At 180 days, 2 of 12 rats had larger nodular aggregates of nonciliated cells which bulged into the lumen. Most rats had a very mild interstitial thickening of alveolar septa in the centriacinar region (LM, SEM, TEM).	Rat	Moore and Schwartz, 1981
1058 1725	0.54 0.88	ND	2, 4, 8, 12, or 48 hr	Severe loss of cilia from TB after 2 hr. TB surface more uniform in height than controls. Necrotic ciliate cells in TB epithelium and free in lumen after 6-12 hr of a 0.88-ppm exposure. Ciliated cell necrosis continued until 24 hr, when little evidence of further cell damage or loss was seen. Only minimal loss of ciliated cells in 0.5-ppm rat group. Nonciliated cells were "resistant" to injury from O_3 and hypertrophic at 72 hr. Damage to the first 2 or 3 alveoli after 0.54-ppm for 2 hr. Type 1 cell "fraying" and vesiculation. Damage was greater after 0.88 for 2 hr. "Basement lamina" denuded. Type 2 and 3 cells resistant. Macrophages accumulated in proximal alveoli. Endothelium appeared relatively normal. Repair started at 20 hr. Type 2 cells divide, cuboidal epithelium lines proximal alveoli where type 1 cells were destroyed. Continued exposure resulted in thickened alveolar walls and tissue surrounding TBs. Exposure for 8-10 hr followed by clean air until 48 hr resulted in a proliferative response (at 48 hr) about equal to that observed after continuous exposure (LM, SEM, TEM).	Rat	Stephens et al., 1974a
1058	0.54		6 months, 24 hr/day	No mention in either the results or discussion of the 6 months at 0.54-ppm group.		

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Refere
1058 1725	0.54 0.88	ND	4 hr to 3 weeks	Ozone-exposed lungs heavier and larger than controls. Increased centriacinar macrophages. Hyperplasia of distal airway epithelium Increased connective tissue elements. Collagen-like strands formed bridges across alveolar openings. Fibrosis more pronounced in 0.88-ppm group.	Rat (month old)	Freeman et al., 1974
1764	0.9 O_3 + 0.9 NO_2		60 days	Respiratory distress during first month. Several rats died. Gross and microscopic appearance of advanced experimental emphysema as produced by NO_2 earlier (Freeman et al., 1972). Ozone potentiated effect of nitrogen dioxide.	Rat (month old)	
490	0.25 O_3 + 2.5 NO_2		6 months	"At 6 months the pulmonary tissue seemed quite normal." Proximal orders of ADs minimally involved.	Rat (month old)	
1176 2548	0.6 1.3	ND	1 or 2 days, 6 hr/day or 7 hr/day	Endothelial cells showed the most disruption. The lining membranes were fragmented. Cell debris was often present in the alveoli as well as the capillaries. Some disorganization of the cytoplasm of the large alveolar corner or wall cells was evident.	Mouse (young)	Bils, 1970
1254	0.64	UV	1 year 8 hr/day	LM and TEM morphometry revealed increased volume density and volumes of RBs which had thicker walls and narrower lumens. Peribronchiolar and perivascular connective tissue was increased by increased inflammatory cells and amorphous extracellular matrix rather than stainable fibers. In RBs cuboidal bronchiolar cells were increased and type 1 cells decreased. The media and intima of small pulmonary arteries were thicker.	Bonnet monkey	Fujinaka et al., 1985
1254 1882 + 5000	0.64 0.96 $(NH_4)_2SO_4$	UV	3, 7 or 14 days	Ammonium sulfate aerosol enhanced the effects of O_3 alone and accelerated the occurrence of these effects. The same numbers of lesions were seen in lungs from rats exposed to either O_3 alone or to O_3 with the aerosol, but the lesions were larger in the latter group. Lesions in the O_3 plus aerosol rat lungs had more inflammatory cells, fibroblasts and stainable collagen fibers.	Rat	Last et al., 1984a
1254	0.64	UV	23 hr/day 3 or 7 days	SEM and TEM, including TEM morphometry, revealed necrosis of ciliated cells, decreased numbers of ciliated cells and loss of cilia. Extracellular space was increased and focal areas of epithelial stratification were seen. Small mucous granule cells were increased and an intermediate cell was described. Regular mucous cells had decreased density and smaller irregularly sized secretory granules which contained only filamentous or granular material. TEM morphometry indicated the most severe lesions occurred at 3 days of exposure and that the epithelium had returned towards normal after 7 days of exposure.	Bonnet monkey	Wilson et al., 1984

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
µg/m³	ppm					
1372 1568	0.7 to 0.8	UV, NBKI	7 days, continuous	*In situ* cytochemical studies of lungs from O_3 exposed and control rats. Ozone-exposed rats had increased acid phosphatase, both in lysosomes and in the cytoplasm, in nonciliated bronchiolar (Clara) cells, alveolar macrophages, type 1 and 2 cells, and fibroblasts.	Rat	Castleman et al., 1973a
1372	0.7	ND	24 hr, continuous	Exposure end: General depletion of cilia from TB surface. Nonciliated cells were shorter and contained fewer dense granules, less SER, and more free ribosomes. Post exposure: (0-4 days) TB returned towards normal.	Rat	Evans et al., 1976a
1568	0.8	UV, NBKI	6, 10, 20 days exposure, 24 hr/day or 20 days exposure + 10 days postexposure	SEM of distal trachea and primary bronchi: 6 days: Cilia of variable length. 10 days: Marked loss of cilia. Very few cells had normal cilia. Some nonciliated cells were in clusters and had wrinkled corrugated surfaces. 20 days: Similar to 10 days. 10 days postexposure: Cilia nearly normal. Clusters of nonciliated cells were present and elevated above the surface. Clusters of nonciliated cells were interpreted as proliferative changes.	Mouse (Swiss Webster)	Ibrahim et al., 1980
1568	0.8	UV, NBKI	7 days, continuous with samples at 6, 24, 72 and 168 hr.	Exposure-related epithelial changes. TB cell populations changed after O_3 exposure; fewer ciliated and more nonciliated secretory cells.	Rat	Lum et al., 1978
1568	0.8	UV, NBKI	4, 8, 12, 18, 26, 36, 50, and post 48 and 168 hr, continuous	Degeneration and necrosis of RB type 1 cells predominates from 4-12 hr. Type 1 cell most sensitive of RB epithelial cells. Labeling index highest at 50 hr. Mostly cuboidal bronchiolar cells but some type 2 cells. Bronchiolar epithelium hyperplastic after 50 hr exposure, which persisted following 7 days postexposure. Intraluminal macrophages increased during exposure, but marked clusters of K cells at 26-36 hr.	Monkey (Rhesus)	Castleman et al., 1980

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
1568	0.8	UV, NBKI	3 days, continuous 0, 2, 6, 9, 16, and 30 days post-exposure; 2nd 3 days continuous after 6, 13, and 27 days postexposure	1st Exposure: TB epithelium flattened and covered with debris. Ciliated cells either unrecognizable or had shortened cilia. The type of most epithelial cells could not be determined. Proximal alveoli had clumps of macrophages and cell debris. Type 2 cells lined surfaces of many proximal alveoli. Occasionally, denuded basal lamina or type 1 cell swelling. Postexposure: 6 days: Most obvious lesions were not present. TB epithelium had usual pattern. Clumps of macrophages had cleared from the lumen. Most proximal alveoli lined by normal type 1 and 2 cells. 30 days: Lungs indistinguishable from controls. 2nd 3-Day Exposure: 6 or 27 days after the end of the 1st. Lesions same as 1st exposure.	Rat (Sprague-Dawley; 70 days old)	Plopper et al., 1978
1666	0.85 Similar exposure regimen for 14 ppm NO_2, but not mixtures.	ND	1, 2, 3 days, continuous	Birth to weaning at 20 days: "Very little indication of response" or "tissue nodules" with dissecting microscope. 12 days: NO_2 lesions but no O_3 lesions. 22 days old: O_3, loss of cilia, hypertrophy of TB cells, tendency towards flattening of luminal epithelial surface. 32 days old: O_3, loss of cilia, and significant hypertrophy of TB epithelial cells. 21 days old and older: Alveolar injury, including sloughing to type 1 cells resulting in bare basal lamina. Response plateau is reached at 35 days of age.	Rat (Sprague-Dawley; 1, 5, 10, 15, 20, 25, 30, 35, and 40 days old)	Stephens et al., 1978
1960	1.0	NBKI	~60 weeks, ~5 days/week, 6 hr/day (268 exposures)	Chronic injury occurred in the lungs of each species of small animal. The principal site of injury was in the terminal airway, as manifested by chronic bronchiolitis and bronchiolar wall fibrosis resulting in tortuosity and stenosis of the passages.	Mouse, Hamster, Rat	Stokinger et al., 1957

TABLE 9-1. MORPHOLOGICAL EFFECTS OF OZONE (continued)

Ozone concentration µg/m^3	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
1960 to 5880 1960 1960 1960 3920 5880	1.0 to 3.0 1.0 1.0 1.0 2.0 3.0	ND	18 months, 8-24 hr/day A = 8 hr/day B = 16 hr/day C = 24 hr/day D = 8 hr/day E = 8 hr/day	Result: A 1 ppm, 8 hr/day: Minimal fibrosis occasionally and randomly in the periphery of an alveolar duct. A few "extra" macrophages in central alveoli. B 1 ppm, 16 hr/day: Occasional fibrous strands in some alveolar openings of RBs and ADs. A few more "extra" macrophages. C 1 ppm, 24 hr/day: More extensive fibrosis of centriacinus. Thickened AD walls. More "extra" macrophages. Sporadic hyperplasia of epithelium of RB and AD. D & E More fibrosis. Epithelial hyperplasia and squamous metaplasia.	Dog	Freeman et al., 1973
1960 and 3920	1.0 and 2.0 Also mixtures with H_2SO_4	CHEM, NBKI	2 or 7 days, 6 hr/day	Results: O_3 alone: Lesions limited to centriacinus. 1 ppm: Hypertrophy and hyperplasia of TB epithelium. Centriacinar alveoli had increased type 2 cells, increased macrophages, and thickened walls. Some edema in all animals. Lesions less severe at 7 days than at 2 days. This adaptation was more rapid in rats than guinea pigs. 2 ppm: Same plus loss of cilia in bronchi. O_3 plus H_2SO_4 No additive or synergistic morphological changes.	Rat and Guinea pig	Cavender et al., 1977

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence); NBKI = neutral buffered potassium iodide; UV = UV photometry; ND = not described.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] Abbreviations used: LM = light microscope; EM = electron microscope; SEM = scanning electron microscope; TEM = transmission electron microscope; PAM = pulmonary alveolar macrophage; RB = respiratory bronchiole; TB = terminal bronchiole; AD = alveolar duct; IAS = interalveolar septa; LDH = lactic dehydrogenase;

SER = smooth endoplasmic reticulum; RER = rough endoplasmic reticulum.

The lower or intrathoracic conducting airways include the thoracic trachea, bronchi, and bronchioles. Species variation of lower airway structure is large, as recorded at the NIH workshop on comparative biology of the lung (National Institutes of Health, 1983). The thoracic trachea and bronchi have epithelial and subepithelial tissues similar to those of the upper conducting airways. In bronchioles, the epithelium does not contain mucous (goblet) cells, but in their place are specialized nonciliated bronchiolar epithelial cells, which in some species can appropriately be called "Clara" cells. Subepithelial tissues are sparse and do not contain glands.

The ciliated cell is the cell in the upper and lower conducting airways in which morphological evidence of damage is most readily seen. This cell is primarily responsible for physical clearance or removal of inhaled foreign material from conducting airways of the respiratory system (see Section 9.3.4). The effects of ozone on this cell type, which is distributed throughout the length of conducting airways, are detected through various physiological tests and several types of morphological examination (Kenoyer et al., 1981; Oomichi and Kita, 1974; Phalen et al., 1980; Frager et al., 1979; Abraham et al., 1980).

The other portion of the respiratory system directly damaged by inhalation of O_3 is the junction of the conducting airways with the gaseous exchange area. The structure and cell makeup of this junction varies with the species. In man, the most distal conducting airways, the terminal bronchioles, are followed by several generations of transitional airways, the respiratory bronchioles, which have gas exchange areas as a part of their walls. In most of the species used for experimental exposures to O_3, (i.e., mouse, rat, guinea pig, and rabbit), the terminal bronchioles are followed by alveolar ducts rather than respiratory bronchioles. The only common experimental animals with respiratory bronchioles are the dog and monkey, and they have fewer generations of nonrespiratory bronchioles than does man as well as differences in the cells of the respiratory bronchioles (National Institutes of Health, 1983).

9.3.1.1.1 Airways

9.3.1.1.1.1 Upper airways (nasal cavity, pharynx, and larynx).

The effects of O_3 on the upper extrathoracic airways have received little attention. The effect of upper airway scrubbing on the level of O_3 reaching the more distal conducting airways has been studied in rabbits and guinea pigs (Miller et al., 1979). They demonstrated removal of approximately 50 percent of ambient concentrations between 196 and 3920 μg/m^3 (0.1 and 2.0 ppm). Earlier, Yokoyama and Frank (1972) studied nasal uptake in dogs. They found uptake to vary with flow rate as well as with O_3 concentration. At 510 to 666 μg/m^3 (0.26 to 0.34 ppm) of O_3, the uptake at low flows of 3.5 to 6.5 L/min was 71.7 ± 1.7 percent, and at high flow rates of 35 to 45 L/min the uptake was 36.9 ± 2.7 percent. At 1529 to 1568 μg/m^3 (0.78 to 0.8 ppm), the uptakes at low and high flows were 59.2 ± 1.3 percent and 26.7 ± 2.1 percent, respectively. The scrubbing effect of the oral cavity was significantly less at all concentrations and flow rates studied. Species variations in uptake by the nasal cavity probably relate to species differences in the complexity and surface areas of the nasal conchae and meatuses (Schreider and Raabe, 1981).

No studies of the effects of O_3 on the nasal cavity were found, but two references to articles in the Japanese literature were cited by Ikematsu (1978). At least one study of the morphological effects of ambient levels of O_3 on the nasal cavity of nonhuman primates is in progress, but not published.

The effects of 392, 1960, and 9800 μg/m^3 (0.2, 1.0, and 5.0 ppm) of O_3 on the tonsils, the primary lymphoreticular structures of the upper airways, were studied. In palatine tonsils from rabbits exposed to 392 μg/m^3 (0.2 ppm) of O_3 continuously for 1 and 2 weeks, Ikematsu (1978) reported epithelial detachment and disarrangement and a slight cellular infiltration. The significance of these observations to the function of immune mechanisms in host defense is unknown.

9.3.1.1.1.2 Trachea. Tracheal epithelial lesions have been described in several species following exposure to less than 1960 μg/m^3 (1 ppm) of O_3. Boatman et al. (1974) exposed anesthetized, paralyzed cats to 510, 980, or 1960 μg/m^3 (0.26, 0.50, or 1 ppm) of O_3 via an endotracheal tube for 4.7 to 6.6 hr. This exposure technique bypassed the nasal cavity, resulting in higher tracheal concentrations than in usual exposures. They reported desquamation of ciliated epithelium at 1960 μg/m^3 (1 ppm) of O_3, but none at 510 or 980 μg/m^3 (0.26 or 0.5 ppm).

In rats exposed to 960 or 1568 μg/m^3 (0.5 or 0.8 ppm) of O_3, 8 or 24 hr/day for 7 days, Schwartz et al. (1976) described focal areas of the trachea in which the cilia were reduced in density and were of variable diameter and length. Mucous cells appeared to have been fixed in the process of discharging mucigen droplets. These changes were more easily seen with the scanning electron microscope (SEM) and were not obvious in rats exposed to 392 μg/m^3 (0.2 ppm) of O_3 for the same times. Cavender et al. (1977), when using only light microscopy (LM), studied tracheas from rats and guinea pigs exposed for 7 days to 1960 or 3920 μg/m^3 (1 or 2 ppm) of O_3 or sulfuric acid (H_2SO_4) or both. The article does not state the hours of exposure per day. Tracheal lesions, which consisted of reduced numbers of cilia and goblet cells, were reported only for guinea pigs exposed to O_3. Animals exposed to both pollutants had lesions similar to those exposed to O_3 alone.

By using SEM and transmission electron microscopy (TEM), Castleman et al. (1977) described shortened and less dense cilia in tracheas from bonnet monkeys exposed to 392 or 686 μg/m^3 (0.2 or 0.35 ppm), 8 hr/day for 7 days. Lesions occurred as random patches or longitudinal tracts. In these areas, the nonciliated cells appeared to be more numerous. The TEM study revealed that cells with long cilia had the most severe cytoplasmic changes, which included dilated endoplasmic reticulum, swollen mitochondria, and condensed nuclei, some of which were pyknotic. In lesion areas, evidence of ciliogenesis was seen in noncilated cells with a microvillar surface. Mucous cells did not appear to be significantly altered, but some had roughened apical surfaces. The changes were more variable and less severe in the 392 μg/m^3 (0.2 ppm) group. More extensive and severe lesions of similar nature were seen in tracheas from rhesus monkeys exposed to 980 or 1568 μg/m^3 (0.5 or 0.8 ppm) of O_3 in the same exposure regimen (Dungworth et al., 1975b; Mellick et al., 1977).

Wilson et al. (1984) evaluated the response of the tracheal epithelium from bonnet monkeys exposed continuously for 3 or 7 days to 1254 μg/m^3 (0.64 ppm) O_3 using SEM, quantitative TEM, and autoradiography. Extracellular space was increased and foci of stratified epithelium were reported. Changes in ciliated cells were generally similar, but more severe, than those reported by Castleman et al. (1977). These changes were more severe at 3 days than at 7 days when both the volume percentage and population density had returned to control values. They also reported fewer and smaller granules in regular, as opposed to small-mucous-granule (SMG), mucous cells. These granules also lacked the biphasic appearance of those seen in control monkeys. At both time periods SMG cells in exposed monkeys were more numerous and had greater numbers of granules than controls.

After mice were exposed to 1568 μg/m^3 (0.8 ppm) of O_3 24 hr/day for 6, 10, or 20 days and for 20 days followed by a 10-day postexposure period during which the animals breathed filtered air, the surface of the tracheas was examined by SEM by Ibrahim et al. (1980). Short and normal-length cilia were seen at 6 days, but at 10 and 20 days, a marked loss of cilia and few normal cilia were seen. Some of the nonciliated cells occurred as clusters and had wrinkled or corrugated surfaces. After the mice breathed filtered air for 10 days, the surface morphology of the cilia returned to near normal, but the clusters of nonciliated cells were still present. Earlier, Penha and Werthamer (1974) observed metaplasia of the tracheal epithelium from mice exposed to high concentrations of O_3 (4900 $\mu g/m^3$, 2.5 ppm) for 120 days. After the mice breathed clean air for 120 days, the metaplasia disappeared, and the epithelium had a nearly normal frequency of ciliated and nonciliated cells.

9.3.1.1.1.3 Bronchi. Bronchial lesions were studied in many of the same animals as those whose tracheal lesions are described above and were reported as generally similar to the tracheal lesions. At low concentrations (Castleman et al., 1977), lesions tended to be more severe in the trachea and proximal bronchi than in distal bronchi or in the next segment of the conduction airways, the nonalveolarized bronchioles. Eustis et al. (1981) reported lesions in lobar, segmental, and subsegmental bronchi from bonnet monkeys exposed to 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 8 hr/day for 7, 28, or 90 days. Lesions at 7 days were similar to those previously described by Mellick et al. (1977) and Castleman et al. (1977), as summarized above. At 28 and 90 days, lesions were not readily apparent by LM, but extensive damage to ciliated cells was seen using SEM. Uniform shortening and reduced density of cilia were seen in linear, parallel arrays. In these areas, the numbers of cells with a flat surface covered by microvilli increased. Wilson et al. (1984) reported similar changes in primary bronchi from bonnet monkeys exposed to 1254 $\mu g/m^3$ (0.64 ppm) O_3 continuously for 3 or 7 days.

Sato et al. (1976a,b) studied bronchi from vitamin E-deficient and control rats exposed to 588 $\mu g/m^3$ (0.3 ppm) of O_3 3 hr/day for up to 16 days. Using LM, they did not see bronchial lesions with asymmetrical swelling and surface roughness, which were obvious with SEM. The observations of Sato et al. (1976a) support the concept that lesions in conducting airways are best seen with SEM and that LM tends to underestimate damage to these ciliated airways. In these short-term studies, lesions were more prominent in vitamin E-deficient rats. This is in contrast to later studies in which Sato et al. (1978, 1980) exposed vitamin E-deficient and supplemented rats to 588 $\mu g/m^3$ (0.3 ppm) of O_3 3 hr/day, 5 days/week for 7 months following which they did not see clear differences due to vitamin E with SEM or TEM.

Yokoyama et al. (1984) studied effects of O_3 on "middle-sized bronchi" of rats fixed by immersion rather than infusion via the airways. They compared the effects of 3 hr/day exposures to 1960 $\mu g/m^3$ (1.0 ppm) O_3 for 30 days with those following 980 $\mu g/m^3$ (0.5 ppm) O_3 6 hr/day for 60 days. They reported increased mucus and irregular loss of cilia, especially on the projections of mucosal folds typical of this type of fixation. Changes were less severe in the 0.5 ppm group.

9.3.1.1.1.4 Bronchioles. There are two types of bronchioles with similar basic structure: those without alveoli in their walls (i.e., nonalveolarized) and those with alveoli opening directly into their lumen (respiratory bronchioles). Man and the larger experimental animals (e.g., nonhuman primates and dogs) have both nonrespiratory and respiratory bronchioles, whereas most of the smaller experimental animals (e.g., mice, rats, and guinea pigs) have only nonrespiratory bronchioles. Because the types, functions, and lesions of epithelial cells are different, these two types of bronchioles will be discussed separately.

Nonrespiratory bronchioles are conducting airways lined by two principal types of epithelial cells: the ciliated and nonciliated bronchiolar cells. The latter cell is frequently called the Clara cell. Although man and most animals have several generations of nonrespiratory bronchioles, some nonhuman primates have only one (Castleman et al., 1975). The last-generation conducting airway before the gas exchange area of the lung is the terminal bronchiole. Terminal bronchioles may end by forming respiratory bronchioles, as in man, monkeys, and dogs, or by forming alveolar ducts, as in mice, rats, and guinea pigs. The acinus, the functional unit of the lung, extends distally from the terminal bronchiole and includes the gas exchange area supplied by the terminal bronchiole and the vessels and nerves that service the terminal bronchiole and its exchange area.

A major lesion due to O_3 exposure occurs in the central portion of the acinus, the centriacinar region, which includes the end of the terminal bronchiole and the first few generations of either respiratory bronchioles or alveolar ducts, depending on the species. The centriacinar region is the junction of the conducting airways with the gas exchange tissues. The O_3 lesion involves both the distal portion of the airway and the immediately adjacent alveoli, the proximal alveoli. In animals with respiratory bronchioles, the lesion is a respiratory bronchiolitis. Regardless of species differences in structure, the lesion occurs at the junction of the conducting airways with the gas exchange area.

Terminal bronchiolar lesions in rats due to inhalation of $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1.0 ppm) of O_3 for 2 hr to 1 week have been described by Stephens et al. (1974a), Evans et al. (1976a,c), Schwartz et al. (1976), and others (Table 9-1). These changes were recently reviewed by Evans (1984). Ciliated cells are the most damaged of the airway cells, and fewer of them are found in the bronchiolar epithelium of exposed animals. Those ciliated cells present tend to have cilia with focal blebs and blunt ends. Damaged ciliated cells are replaced by nonciliated bronchiolar (Clara) cells (Evans et al., 1976a; Lum et al., 1978), which become hyperplastic. The typical projection of the nonciliated or Clara cell into the lumen is reduced, and the luminal surface has increased granularity. The reduction in projection height appears to be due to a reduction in agranular (smooth) endoplasmic reticulum (Schwartz et al., 1976). Many ciliated cells contain basal bodies and precursors of basal bodies indicative of ciliogenesis (Schwartz et al., 1976). The few brush cells present in nonrespiratory bronchioles appeared normal (Schwartz et al., 1976). The lesions were more severe in higher generation, more distal nonrespiratory bronchioles than in the lower generation, more proximal nonrespiratory bronchioles.

In an earlier study, Freeman et al. (1974) exposed month-old rats continuously to 1058 or 1725 $\mu g/m^3$ (0.54 or 0.88 ppm) of O_3 for 4 hr to 3 weeks. In addition to the centriacinar accumulations of macrophages and the hyperplasia of the distal airway epithelium, they reported an increase in connective tissue elements and collagen-like strands which formed bridges across alveolar openings. In the 1725-$\mu g/m^3$ (0.88-ppm) O_3 group, the fibrosis was pronounced and sometimes extended into terminal bronchioles. In the same study, Freeman et al. (1974) exposed month-old rats to a mixture of 1764 $\mu g/m^3$ (0.9 ppm) O_3 and 1690 $\mu g/m^3$ (0.9 ppm) nitrogen dioxide, or to a mixture of 490 $\mu g/m^3$ (0.25 ppm) O_3 and 4700 $\mu g/m^3$ (2.5 ppm) nitrogen dioxide. After 60 days of exposure to the 0.9/0.9 mixture, Freeman et al. (1974) reported that "both grossly and microscopically, the appearance of the lungs was characteristic of advanced experimental emphysema," referring to earlier nitrogen dioxide exposures (Freeman et al., 1972). Freeman et al. (1974) did not report emphysema in rats exposed to O_3 alone, only in those rats exposed to the 0.9/0.9 mixture. The topic of emphysema is discussed later (Section 9.3.1.4.2).

Results differ in four studies of long-term (3- to 6-month) exposures of rats to < 1960 $\mu g/m^3$ (<1.0 ppm) for 6 or 8 hr/day. The differences appear to be due at least in part to the methods used to evaluate the bronchioles. When using only LM to study effects in rats exposed for 6 months to 980 μg/m^3 (0.5 ppm) for 6 hr/day, Cavender et al. (1978) did not find exposure-related lesions. Barr (1984) exposed rats to 1862 μg/m^3 (0.95 ppm) O_3 8 hr/day for 90 days. He examined the bronchioles with LM, TEM, and SEM. Using SEM he reported loss of apical projections of nonciliated cells and loss of both density and height of cilia. Boorman et al. (1980) and Moore and Schwartz (1981) reported significant bronchiolar lesions following exposure to 980 or 1568 μg/m^3 (0.5 or 0.8 ppm) of O_3 8 hr/day for 90 or 180 days. In both studies, loss or shortening of cilia and flattening of the luminal projections of nonciliated bronchiolar cells were observed in terminal bronchioles at each time period, including the end of exposure at 90 or 180 days. Clusters of four to six nonciliated bronchiolar cells, in contrast to dispersed individual cells in controls, were seen at 90 days in both studies, but not at 180 days. However, in 2 of the 12 rats exposed 180 days, larger nodular aggregates of hyperplastic cells projected into the bronchial lumen. After 50, 90, and 180 days of exposure, the nature of the junction between the terminal bronchiole and the alveolar ducts changed from the sharp demarcation seen in controls to a gradual transition with the appearance of a respiratory bronchiole. The presence of this change in distal airway morphology was confirmed by Barr (1984). Both ciliated and nonciliated bronchiolar cells were seen on thickened tissue ridges between alveoli. This change could result from either alveolarization of the terminal bronchiole or bronchiolization of alveolar ducts. Although this change in the airway morphology persisted, the changed segment reduced in length after the 180-day-exposed rats had breathed filtered air for 62 postexposure days. The addition of 1 to 10 mg/m^3 H_2SO_4 to these concentrations of O_3 for the same exposure times did not potentiate the lesions seen in the O_3-alone rats (Moore and Schwartz, 1981; Cavender et al., 1978; Juhos et al., 1978).

Ozone-induced bronchiolar lesions in mice are similar to those seen in rats, but the hyperplasia of the nonciliated cells is more severe (Zitnik et al., 1978); Ibrahim et al., 1980). Following high concentrations (4900 μg/m^3, 2.5 ppm), Penha and Werthamer (1974) noted persistence (unchanged in frequency or appearance) or micronodular hyperplasia of noncilated bronchiolar cells for 120 postexposure days following 120 days of exposure. At lower O_3 levels (980 or 1568 μg/m^3, 0.5 or 0.8 ppm), the hyperplasia was pronounced (Zitnik et al., 1978; Ibrahim et al., 1980). Ibrahim et al. (1980) noted hyperplastic clusters of nonciliated cells 10 days after exposure but did not make observations after longer postexposure periods.

Guinea pigs were exposed by Cavender et al. (1977, 1978) continuously to 1 or 2 ppm of O_3 for 2 or 7 days in acute studies and to 980 $\mu g/m^3$ (0.5 ppm) 6 hr/day, 5 days/week for 6 months. Morphological effects were studied only by LM. The acute, higher concentration distal-airway lesions were similar to those seen in rats and included loss of cilia and hyperplasia of nonciliated cells. The authors reported that the long-term, lower concentration lesions were more severe in guinea pigs than those in rats exposed to a similar regimen. The lesions were no more severe in guinea pigs exposed to a combination of H_2SO_4 aerosol and O_3.

9.3.1.1.2 Parenchyma. Ozone does not affect the parenchyma in a uniform manner. The centriacinar region (i.e., the junction of the conducting airways with the gas exchange area) is the focus of damage, and no changes have been reported in the peripheral portions of the acinus.

9.3.1.1.2.1 Respiratory bronchioles. Respiratory bronchioles are the focus of effects, because they are the junction of the conducting airways with the gas exchange area. However, not all animals have respiratory bronchioles. They are well developed in man but are absent or poorly developed in the common laboratory animals frequently used for O_3 study, with the exception of dogs (Freeman et al., 1973) and macaque monkeys (Mellick et al., 1975, 1977; Dungworth et al., 1975b; Castleman et al., 1977, 1980; Eustis et al., 1981). Short-term exposures of monkeys to 392, 686, 980, or 1568 $\mu g/m^3$ (0.2, 0.35, 0.5, or 0.8 ppm) of O_3 8 hr/day for 7 days resulted in damage to type 1 cells and hyperplasia of nonciliated bronchiolar cells, which were visible by either light or electron microscopy. At lower concentrations, these lesions were limited to the proximal, lower generation respiratory bronchioles. At higher concentrations, the lesions extended deeper into the acinus. The lesions were focused at the junction of the conducting airways with the gas exchange area and extended from that junction with increasing O_3 concentration.

The pathogenesis of the lesions due to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for periods up to 50 hr of exposure was studied quantitatively by Castleman et al. (1980). Damage to type 1 cells was very severe following 4, 8, and 12 hr of exposure. Type 1 cell necrosis, which resulted in bare basal lamina, reached a maximum at 12 hr. The absolute and relative numbers of these cells decreased throughout the exposure. Only a few type 2 cells had mild degenerative changes and only at 4 or 12 hr of exposure. Cuboidal bronchiolar cells had mild degenerative changes, swollen mitochondria, and endoplasmic reticulum at all times except 18 hr. Both cuboidal bronchiolar and type 2 cells functioned as stem cells in renewal epithelium, and both contributed to the hyperplasia seen at the latter exposure times. The inflammatory exudate included both fibrin and a variety of leukocytes in the early phases. In the latter phases, the inflammatory cells were almost entirely macrophages. Inflammatory cells were also seen in the walls of respiratory bronchioles and alveoli opening into them. These lesions were not completely resolved after 7 days of filtered air breathing.

Monkeys exposed to 960 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 8 hr/day for 90 days had a low-grade respiratory bronchiolitis characterized by hypertrophy and hyperplasia of cuboidal bronchiolar cells and intraluminal accumulation of macrophages (Eustis et al., 1981). After the 90-day exposure, the percentage of cuboidal respiratory bronchiolar epithelial cells was 90 percent rather than the 60 percent found in controls. Intraluminal cells, mostly macrophages, reached a maximum of a thirty-seven-fold increase after 7 days of exposure. Their numbers decreased with continued exposure, but at 90 days of exposure, they were still sevenfold higher than those of controls. This study did not include a postexposure period.

Fujinaka et al. (1985) exposed adult male bonnet monkeys to 1254 $\mu g/m^3$ (0.64 ppm) O_3 8 hr/day for one year. They reported significantly increased volume of respiratory bronchioles which had smaller internal diameters. Respiratory bronchiolar walls were thickened by epithelial hyperplasia and increased peribronchiolar connective tissue. Several small nodules of hyperplastic and hypertrophied cuboidal bronchiolar cells were reported near the openings of alveoli into the respiratory bronchiole. The authors interpret the morphometry of respiratory bronchioles as indicating an extension of bronchiolar epithelium into airways which were formerly alveolar ducts similar to the formation of respiratory bronchioles reported in O_3-exposed rats by Boorman et al. (1980) and Moore and Schwartz (1981).

In an earlier study, Freeman et al. (1973) exposed female beagle dogs to 1960 $\mu g/m^3$ (1 ppm) of O_3 8, 16, or 24 hr/day for 18 months. Dogs exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 for 8 hr/day had the mildest lesions, which were obvious only in the terminal airway and immediately adjacent alveoli, where minimal fibrosis and a few extra macrophages were seen. More fibrous strands and macrophages were seen in centriacinar areas of lungs from dogs exposed 16 hr/day. Lungs from dogs exposed 24 hr/day had terminal airways distorted by fibrosis and thickened by both fibrous tissue and a mononuclear cell infiltrate. Relatively broad bands of connective tissue were reported in distal airways and proximal alveoli. Epithelial hyperplasia was seen sporadically in the bronchiolar-ductal zone. Other dogs in that study exposed to 3920 or 5880 μg/m^3 (2 or 3 ppm) of O_3 8 hr/day for the same period had more severe fibrosis, greater accumulations of intraluminal macrophages, and areas of both squamous and mucous metaplasia of bronchiolar epithelia.

9.3.1.1.2.2 Alveolar ducts and alveoli. Alveoli in the centriacinar region, but not those at the periphery of the acinus, are damaged by ambient levels of O_3 (Stephens et al., 1974b; Schwartz et al., 1976; Mellick et al., 1977; Crapo et al., 1984). The lesion is characterized by the destruction of type 1 alveolar epithelial cells exposing the basal lamina; an accumulation of inflammatory cells, especially macrophages; hyperplasia of type 2 alveolar epithelial cells that recover the denuded basal lamina; and thickening of the interalveolar septa. In animals with respiratory bronchioles (e.g., dog, monkey) the alveoli involved at low concentrations are those opening directly into the respiratory bronchiolar lumen of low-generation respiratory bronchioles (Dungworth et al., 1975b). As the concentration is increased, the lesions include alveoli attached to but not, seemingly, opening into the respiratory bronchioles and extending distally to higher-generation respiratory bronchioles (Mellick et al., 1977). In monkeys exposed to 1568 μg/m^3 (0.8 ppm) of O_3, alveoli opening into alveolar ducts are minimally involved (Mellick et al., 1977). In animals that lack respiratory bronchioles (e.g., rat, mouse, guinea pig) the alveoli involved open into or are immediately adjacent to the alveolar ducts formed by the termination of the terminal bronchiole.

In the centriacinar region of animals which lack respiratory bronchioles, damage to type 1 cells has been reported as early as 2 hours following exposure to 980 μg/m^3 (0.5 ppm) O_3 using LM (Stephens et al., 1974a). While not an O_3 concentration studied comprehensively or illustrated in that article, the same authors comment in the results section of their report that TEM evaluation of rats exposed to 392 μg/m^3 (0.2 ppm) O_3 for 2 hours revealed "...considerable damage and loss of type 1 cells from proximal alveoli..." Recovering of denuded basal lamina by type 2 cells has been reported to start as early as 4 hr (Stephens et al., 1974b). The type 2 cell labeling index following tritiated thymidine reached a maximum at 2 days of continuous exposure to either 686 or 980 μg/m^3 (0.35 or 0.5 ppm) of O_3 (Evans et al., 1976b). Although the labeling index decreases as the exposure continues (Evans et al., 1976b), clusters of type 2 cells and cells intermediate between types 2 and 1 were reported following 90 days of exposure to 1568 μg/m^3 (0.8 ppm) by Boorman et al. (1980). They interpreted these intermediate cells as due to delay or arrest of the transformation from type 2 to type 1 epithelial cells. Type 1 cell damage and occasional sloughing were observed by Barry et al. (1983) in newborn rats exposed to 490 $\mu g/m^3$ (0.25 ppm) of O_3 12 hr/day for 6 weeks. By using LM and TEM morphometric techniques, these authors also found that centriacinar alveoli also had more type 1 and 2 epithelial cells and macrophages. The type 1 cells were smaller in volume, covered less surface, and were thicker. Evans et al. (1985) studied this effect and suggested that O_3 tolerance exists when the surface area of a cell is small enough so that antioxidant mechanisms contained in that volume can protect it from damage. Sherwin et al. (1983) found increased numbers of lactate dehydrogenase (LDH)-positive cells, presumed to be type 2 alveolar epithelial cells, by automated LM morphometry of lungs from mice exposed to 588 $\mu g/m^3$ (0.3 ppm) of O_3 for 6 weeks. Moore and Schwartz (1981) reported nonciliated bronchiolar cells lined some alveoli opening into the transformed airways located between terminal bronchioles and alveolar ducts of rats exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 8 hr/day for 180 days. Sulfuric acid aerosol did not potentiate this lesion.

The inflammatory cell response appears to occur immediately following or concurrent with the type 1 cell damage and has been reported in monkeys as early as after 4 hr of 1568 $\mu g/m^3$ (0.8 ppm) of O_3 exposure (Castleman et al., 1980). In rats, the numbers of inflammatory cells per centriacinar alveolus appear to be related to O_3 concentration, at levels between 392 and 1568 $\mu g/m^3$ (0.2 and 0.8 ppm), during 7-day (Brummer et al., 1977) and 90-day exposures (Boorman et al., 1980). Using the same technique, Moore and Schwartz (1981) found statistically significant increases after 3, 50, 90, and 180 days of exposure 8 hr/day to 980 $\mu g/m^3$ (0.5 ppm) of O_3 and after 62 days of filtered air following 180 days of exposure. The addition of 1 mg/m^3 H_2SO_4 aerosol did not result in larger increases. In monkeys, the intensity of the response was greater than in rats exposed to the same O_3 concentration (1568 $\mu g/m^3$, 0.8 ppm) in the same regimen (8 hr/day) for 7, 28, or 90 days (Eustis et al., 1981). Although in both species the numbers of inflammatory cells per alveolus decreased with increasing length of exposure, the decrease was not as rapid in the monkey as in the rat (Eustis et al., 1981).

Several investigators (Boorman et al., 1980; Moore and Schwartz, 1981; Fujinaka et al., 1985; Barr, 1984) have presented evidence of bronchiolization of airways which were previously alveolar ducts; i.e., bronchial epithelium replaces the type 1 and 2 alveolar epithelium typical of alveolar ducts.

This phenomenon is most easily seen and quantitated in species which normally do not have respiratory bronchioles or have one very short generation of them. In these species, airways with the characteristics of respiratory bronchioles are seen between the terminal bronchioles and the alveolar ducts of exposed but not control animals (Boorman et al., 1980; Moore and Schwartz, 1981; Barr, 1984). In species which normally have several generations of respiratory bronchioles, bronchiolization is detected morphometrically by increases in the volume fraction or total volume of respiratory bronchioles. Using morphometric techniques, bronchiolization was reported in nonhuman primates exposed to 1254 $\mu g/m^3$ (0.64 ppm) O_3 8 hr/day for one year (Fujinaka et al., 1985).

The interalveolar septa of centriacinar alveoli are thickened following exposure to ambient concentrations of O_3. After 7 days of continuous exposure, the thickening was attributed to eosinophilic hyaline material and mononuclear cells (Schwartz et al., 1976). Loose arrangements of cells and extracellular materials suggested separation by edema fluid. Castleman et al. (1980) also reported edema of interalveolar septa of monkeys exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for 4 to 50 hr. Boorman et al. (1980) used morphometric techniques on electron micrographs to quantitatively evaluate the thickness of centriacinar interaveolar septa. The arithmetic mean thickness was increased in rats exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 8 hr/day for 20 or 90 days. The increased total thickness was due to thicker interstitium. Although several components could contribute to this increased thickness, the subjective impression was one of a mild interstitial fibrosis. Crapo et al. (1984) made a more comprehensive morphometric study of centriacinar interalveolar septa from young adult rats exposed to 490 $\mu g/m^3$ (0.25 ppm) O_3 12 hr/day for 6 weeks. They reported significant increases in tissue thickness and suggested that the increased thickness was due to significant increases in all cell types except type 2 cells, and to increased interstitium.

Moore and Schwartz (1981), after exposing rats to 980 $\mu g/m^3$ (0.5 ppm) of O_3 24 hr/day for 180 days, reported very mild interstitial thickening of centriacinar interalveolar septa, which they concluded was due to collagen. Earlier, Freeman et al. (1973) morphologically demonstrated fibrosis in beagle dogs exposed to 1960 to 5880 $\mu g/m^3$ (1 to 3 ppm) of O_3 8 to 24 hr/day for 18 months.

In several biochemical studies (see Section 9.3.3.6), Last and colleagues have shown that O_3 is collagenic. In one of these (Last et al., 1979), the biochemical observations were correlated with histological observations of slides stained for collagen and reticulin. Elevated collagen synthesis rates were found at all concentrations and times studied. Mildly increased amounts of collagen were seen morphologically in centriacinar alveolar duct septa from most rats exposed to 980 μg/m^3 (0.5 ppm) of O_3 24 hr/day for 14 or 21 days. More severe lesions were seen in rats exposed to 1568 to 3920 μg/m^3 (0.8 to 2.0 ppm) of O_3 24 hr/day for 7, 14, or 21 days. Last et al. (1983, 1984a) have reported synergistic increases in morphometrically determined volume of centriacinar lesions, inflammatory cells, and "stainable" collagen in rats exposed to a mixture of O_3 and ammonium sulfate aerosols. The 1984 report concerns this synergism in rats exposed to 1254 or 1882 μg/m^3 (0.64 or 0.96 ppm) O_3 and 5 mg/m^3 ammonium sulfate for 3, 7, or 14 days. The results correlate well with biochemically determined apparent collagen synthesis rates (see Section 9.3.3.6).

Two studies address the biologically important question of the morphological effects that follow multiple sequential exposures to O_3 with several days of clean air interspersed between O_3 exposures (i.e., a multiple episode exposure regime). Plopper et al. (1978) exposed rats to 1568 μg/m^3 (0.8 ppm) O_3 continuously for 3 days, held them in the chambers breathing filtered air until postexposure day 6, 13, or 27, when they were again exposed to 1568 μg/m^3 (0.8 ppm) of O_3 continuously for 3 days. Rats were also examined 2, 6, 9, 16, and 30 days after the first O_3 exposure. Lungs from rats breathing filtered air for 9 days after one 3-day exposure had only minimal lesions and after 30 days of filtered air were indistinguishable from controls. When the second 3-day O_3 exposure started 6 or 27 days after the end of the first exposure, the lesions appeared identical to each other and to those seen at the end of the first exposure. Barr (1984) compared lesions in rats exposed to 1862 μg/m^3 (0.95 ppm) O_3 8 hours every day for 90 days with both control rats and with rats exposed to the same concentration 8 hours per day in 5-day episodes followed by 9 days of filtered air repeated 7 times during an 89-day period. The lesions were similar but less severe in the episodically exposed rats.

9.3.1.1.3 Vasculature, blood, and lymphatics. Although edema is the apparent cause of death due to inhalation of high concentrations of O_3, there is very little morphological evidence of pulmonary vascular damage due to $\leq$ 1960 μg/m^3 ($\leq$ 1.0 ppm) of O_3 exposure. Bils (1970) reported capillary endothelial damage in mice less than 1 month of age exposed for 7 hr to 1960 μg/m^3 (1 ppm) of O_3, but this experiment has not been confirmed by others. Boatman et al. (1974)

did demonstrate endothelial damage in cats exposed via an endotracheal tube to 510, 980, or 1960 μg/m^3 (0.26, 0.5, or 1.0 ppm) of O_3 for 4 to 6 hr, but it is not clear whether all exposure levels resulted in endothelial damage. In later studies that used pneumonectomized and control rabbits, Boatman et al. (1983) reported occasional swelling of capillary endothelium in both groups exposed to 784 μg/m^3 (0.4 ppm) of O_3 7 hr/day, 5 days/week for 6 weeks. Stephens et al. (1974b) reported occasional areas of endothelial swelling but concluded "the endothelium remains intact and rarely shows signs of significant injury." Stephens et al. (1974a) reported that "endothelium retained a relatively normal appearance" in rats exposed to 980 or 1764 μg/m^3 (0.5 or 0.9 ppm) of O_3 for 2 to 12 hr or 980 μg/m^3 (0.5 ppm) for up to 6 months. In rats exposed by the usual methods to 980 or 1568 μg/m^3 (0.5 or 0.8 ppm) of O_3 8 or 24 hr/day, centriacinar interalveolar septa had a loose arrangement of cells and extracellular material, indicating separation by edema fluid (Schwartz et al., 1976). These investigators did not find morphological evidence of damage to endothelial cells. Evidence of intramural edema in centriacinar areas was found by Castleman et al. (1980) in monkeys exposed to 1568 μg/m^3 (0.8 ppm) for 4 to 50 hr, but they did not report morphological evidence of vascular endothelial damage.

Arterial lesions have been only rarely reported. P'an et al. (1972) reported increased thickness of the media and intima in pulmonary arteries from rabbits exposed to 784 μg/m^3 (0.4 ppm) O_3 6 hr/day, 5 days/week for 10 months. These rabbits had evidence of intercurrent disease which was more severe in exposed animals. The LM description indicates "some degree of inflammatory infiltrate" in all lungs, and in one exposed rabbit the lesions were so severe that "visualization of the mural framework of the alveoli was difficult." The pulmonary artery media and intima were also significantly thickened in bonnet monkeys exposed to 1254 μg/m^3 (0.64 ppm) O_3 8 hr/day for one year (Fujinaka et al., 1985).

No references to morphological damage of lymphatic vessels were found. This is not surprising, because following nasal inhalation of $\leq$ 1960 μg/m^3 ($\leq$1 ppm) of O_3, blood capillary endothelial damage has not been reported, and edema has been reported only in centriacinar structures. In the more generalized edema that follows exposure to higher concentrations, Scheel et al. (1959) reported perivascular lymphatics were greatly distended.

9.3.1.2 Sequence in which Sites are Affected as a Function of Concentration and Duration of Exposure. The sequence in which anatomic sites are affected appears to be a function of concentration rather than of exposure duration. At sites that are involved by a specific concentration, however, the stages in pathogenesis of the lesion relate to the duration of exposure. Multiple anatomical sites in the conducting and exchange areas of the respiratory system have been studied at sequential time periods in only a few studies. Stephens et al. (1974a), in a study of the short-term effects of several concentrations of O_3, reported finding by LM significant damage to centriacinar type 1 alveolar epithelial cells in rats exposed to 980 μg/m^3 (0.5 ppm) O_3 for 2 hours. Using TEM, they also reported, but did not document by figures, minimal damage to centriacinar type 1 cells in rats exposed to 392 μg/m^3 (0.2 ppm) O_3 for 2 hours. Boatman et al. (1974) found lesions in both the conducting airways and parenchyma of cats exposed to 510, 980, or 1960 μg/m^3 (0.26, 0.5, or 1.0 ppm) via an endotracheal tube for times as short as 4.7 and 6.6 hr. Thus, if there is a time sequence in effect at various sites, it is a short time.

Increasing concentration not only results in more severe lesions, but also appears to extend the lesions to higher generations of the same type of respiratory structure (i.e., deeper into the lung) (Dungworth et al., 1975b). Several investigators who have described gradients of lesions have related them to assumed decreases in concentration of O_3 as it progresses through increasing generations of airways and to differences in protection and sensitivity of cells at various anatomic sites. For the conducting airways, Mellick et al. (1977) reported more severe and extensive lesions in the trachea and major bronchi than in small bronchi or terminal bronchioles of rhesus monkeys exposed to 980 or 1568 μg/m^3 (0.5 or 0.8 ppm) of O_3 8 hr/day for 7 days. For the acinus, they noted the most severe damage in proximal respiratory bronchioles and their alveoli rather than in more distal, higher generation ones. Proximal portions of alveolar ducts were only minimally involved. The predominant lesion was at the junction of the conducting airways with the exchange area. In monkeys, as in man, the proximal respiratory bronchioles, not alveolar ducts, are in the central portion of the acinus. Similar gradients of effects in the conducting airways and the centriacinar region were reported by Castleman et al. (1977), who studied bonnet monkeys exposed to 392 and 686 μg/m^3 (0.2 and 0.35 ppm) of O_3 8 hr/day for 7 days. In the centriacinar region, this gradient is most easily demonstrated by the hyperplasia of cuboidal cells in respiratory bronchioles that extended further distally in monkeys exposed to 686 rather than 392 μg/m^3 (0.35 than 0.2 ppm) of O_3.

The effects of duration of exposure are more complex. In the time frame of a few hours, an early damage phase has been observed at 2 or 4 hr of exposure (Stephens et al., 1974a,b; Castleman et al., 1980). Repair of the damage, as indicated by DNA synthesis by repair cells, occurs as early as 18 hr (Castleman et al., 1980) or 24 hr (Evans et al., 1976b; Lum et al., 1978). Stephens et al. (1974a) reported little change in the extent of damage after 8 to 10 hr of exposure. Full morphological development of the lesion occurs at about 3 days of continuous exposure (Castleman et al., 1980). Damage continues while repair is in progress, but at a lower rate. This phenomenon has been termed adaptation (Dungworth et al., 1975b). When the time frame is shifted from hours to days, severity of the lesion at 7 days differs little between exposures of 8 hr/day and 24 hr/day (Schwartz et al., 1976). When the time frame is again shifted from days to months of daily exposures, the centriacinar lesions diminish in magnitude, but a significant lesion remains (Boorman et al., 1980; Moore and Schwartz, 1981; Eustis et al., 1981).

9.3.1.3. Structural Elements Affected

9.3.1.3.1 Extent of injury to individual cell types. The extent of injury to an individual cell is related to the product of the sensitivity of that cell type and the dose of O_3 delivered to the specific site occupied by that cell. Other factors, e.g. maturity of the individual cell, may also be involved. The dose to an individual cell is determined by the concentration of O_3 at that specific site in the respiratory system and the surface area of the cell exposed to that concentration of O_3. Thus, sensitivity and extent of morphologically detected injury are not the same. While O_3 concentrations at any specific site in the respiratory system can not be determined using the usual analytical methods, the concentration can be estimated using modeling techniques (See Section 9.2). The literature does contain extensive information on the extent of morphologically detected injury to individual cells at specific sites in the respiratory system. That information is reviewed in the following paragraphs. Ciliated cells of the trachea and proximal, lower generation bronchi are subject to more damage than those located in distal, higher generation bronchi or in lower generation bronchioles proximal to the terminal bronchiole (Schwartz et al., 1976; Mellick et al., 1977). Ciliated cells in terminal bronchioles of animals without respiratory bronchioles (i.e., rats) are severely damaged by even low concentrations of O_3 (Stephens et al., 1974a; Schwartz et al., 1976), whereas those in terminal bronchioles of animals with respiratory bronchioles (i.e., monkeys) are much less subject to damage (Castleman et al., 1977; Mellick et al., 1977).

In a similar manner, type 1 alveolar epithelial cells located in the centriacinar region are subject to damage by low concentrations of O_3, but those in the peripheral portions of the acinus appear undamaged by the same or higher concentrations (Stephens et al., 1974a,b; Schwartz et al., 1976; Castleman et al., 1980; Crapo et al., 1984).

Although type 2 alveolar epithelial cells appear to be damaged less by O_3, some type 2 cells in centriacinar locations develop mild lesions detectable with the TEM (Castleman et al., 1980). There is one report of larger than normal lamellar bodies in type 2 cells from rats fed a special basal diet with or without vitamin E supplementation and exposed to 588 $\mu g/m^3$ (0.3 ppm) O_3 3 hr/day for 11 or 16 days (Shimura et al., 1984). Type 2 cells are progenitor cells that recover basal lamina denuded by necrosis or sloughing of type 1 cells and transform (differentiate) into type 1 cells when repairing the centriacinar O_3 lesion (Evans et al., 1976b). Increased DNA synthesis by type 2 cells, as evaluated by autoradiography, may be a very sensitive indicator of O_3 damage.

Although nonciliated cuboidal bronchiolar cells appear less damaged morphologically by O_3 than ciliated cells and are the progenitor cells for replacement of damaged ciliated cells (Evans et al., 1976a,c; Lum et al., 1978), they are a sensitive indicator of O_3 damage (Schwartz et al., 1976). Following exposure to 1960 $\mu g/m^3$ (< 1 ppm) of O_3, their height is reduced and their luminal surface is more granular (Schwartz et al., 1976). The reduction in height appears to be due to a loss of smooth endoplasmic reticulum (Schwartz et al., 1976).

Several investigators report that type 3 alveolar epithelial cells, the brush cells, are not damaged by less than 1 ppm of O_3 (Stephens et al., 1974a; Schwartz et al., 1976). No reports are available of damage to type 3 cells by higher concentrations.

Vascular endothelial cells in capillaries of the interalveolar septa may be damaged much less than earlier reports indicated, because lesions are not described in detailed studies using TEM (Stephens et al., 1974a,b; Schwartz et al., 1976; Mellick et al., 1977; Crapo et al., 1984). In the earlier reports, damaged endothelial cells were those located immediately deep to denuded basal lamina and resulted from sloughing of type 1 epithelial cells in the centriacinar region (Bils, 1970; Boatman et al., 1974). Stephens et al. (1974b) reported occasional areas of endothelial swelling but the endothelium in these areas appeared relatively normal and the capillary bed was intact (Stephens et al., 1974a,b).

Morphological damage to the various types of interstitial cells in the interalveolar septa has not been reported. During O_3 exposure, inflammatory cells migrate into the centriacinar interalveolar septa (Schwartz et al., 1976). Later, more collagen and connective tissue ground substance is found in the interalveolar septa (Moore and Schwartz, 1981). Boorman et al. (1980) reported centriacinar interalveolar septa from rats exposed to 1568 $\mu g/m^3$ (0.8 ppm) O_3 8 hr/day for 20 or 90 days had thicker blood-air barriers which contained more interstitium. Crapo et al. (1984) made a more comprehensive morphometric study of centriacinar interalveolar septa from young adult rats exposed to 490 $\mu g/m^3$ (0.25 ppm) O_3 12 hr/day for 6 weeks. They reported significant increases in tissue thickness and suggested that the increased thickness was due to significant increases in all cell types except type 2 cells, and to increased interstitium.

Mucous-secreting cells in conducting airways appear relatively resistant to O_3. Boatman et al. (1974), in studies of cats exposed to $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1.0 ppm) of O_3 via an endotracheal tube for short periods, did find limited desquamation of these cells, but the authors also observed that most appeared intact and increased in size. Castleman et al. (1977) noted roughened apical surfaces of mucous cells, which appeared to be associated with mucigen droplets near the cell surface, but did not find other alterations in pulmonary mucous cells from monkeys exposed to 392 or 686 $\mu g/m^3$ (0.2 or 0.35 ppm) of O_3 8 hr/day for 7 days. Mellick et al. (1977) mention that mitochondrial swelling and residual bodies seen in ciliated cells were not seen in mucous cells in conducting airways of monkeys exposed to 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 8 hr/day for 7 days. Schwartz et al. (1976), who reported mucigen droplets being released from the apical surfaces of mucous cells and mucous droplets trapped among cilia, did not find changes suggesting damage to organelles in rats exposed to 392, 980, or 1568 $\mu g/m^3$ (0.2, 0.5, or 0.8 ppm) of O_3 8 or 24 hr/day for 7 days. Wilson et al. (1984) reported only minor changes in tracheal mucous cells from bonnet monkeys continuously exposed for 3 or 7 days to 1254 $\mu g/m^3$ (0.64 ppm) O_3. Using TEM they reported more prominent small-mucous-granule (SMG) cells which had more abundant cytoplasm and more specific granules. They speculated that SMG cells may relate to the repair process. In regular mucous cells they reported fewer mucous granules dispersed in more cytoplasm. The mucous granules appeared smaller and differed from controls in that they lacked the typical biphasic appearance and had only filamentous or granular secretory material. No reports of damage to conducting airways other than to ciliated and mucous cells were found.

9.3.1.3.2 Extracellular elements (structural proteins). Although physiologic and biochemical changes following O_3 exposure suggest changes in the extracellular structural elements of the lung, no direct morphological evidence has been given of changes in the extracellular structural elements themselves, in contrast to changes in their location or quantity. These physiologic and biochemical studies are discussed elsewhere in this document (See Sections 9.3.2.2 and 9.3.3.6, respectively). Three studies provide morphological evidence of mild fibrosis (i.e., local increase of collagen) in centriacinar interalveolar septa following exposure to < 1960 $\mu g/m^3$ (< 1 ppm) of O_3 (Last et al., 1979; Boorman et al., 1980; Moore and Schwartz, 1981). Changes in collagen location or amounts, or both, which occur with the remodeling of the distal airways, were reported in two of those studies (Boorman et al., 1980; Moore and Schwartz, 1981). One study (Fujinaka et al., 1985) reported increased connective tissue surrounding respiratory bronchioles from bonnet monkeys exposed to 1254 $\mu g/m^3$ (0.64 ppm) O_3 8 hr/day for one year. This increase was due to increased amorphous extracellular matrix rather than stainable connective tissue fibers. Evidence of more collagen or changes in collagen location is in the report of dogs exposed to 1960 or 5880 $\mu g/m^3$ (1 or 3 ppm) of O_3 for 18 months (Freeman et al., 1973).

9.3.1.3.3 Edema. Morphologically demonstrable alveolar edema, or alveolar flooding--an effect of higher-than-ambient levels of O_3 (Scheel et al., 1959; Cavender et al., 1977)--is not reported after exposures to $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1.0 ppm) of O_3 for short or long exposure periods (Schwartz et al., 1976; Cavender et al., 1978; Mellick et al., 1977; Eustis et al., 1981; Boorman et al., 1980; Moore and Schwartz, 1981). Mild interstitial edema of conducting airways (Mellick et al., 1977) and centriacinar parenchymal structures (Schwartz et al., 1976; Castleman et al., 1980; Mellick et al., 1977) is seen following exposure of monkeys or rats to $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1 ppm) of O_3 for several hours to 1 week. Interstitial edema is not reported following longer-term (i.e., weeks to months) exposure to $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1 ppm) or less (Cavender et al., 1977; Eustis et al., 1981; Boorman et al., 1980; Moore and Schwartz, 1981; Zitnik et al., 1978). Biochemical indicators of edema are described in Section 9.3.3.

9.3.1.4 Considerations of Degree of Susceptibility to Morphological Changes

9.3.1.4.1 Compromised experimental animals. Compromised experimental animals (e.g., those with a special nutritional or immunological condition) in a disease state or of young or old age may respond to O_3 exposure with greater, lesser, or a different type of response than the normal, healthy, young adult animals usually studied. Some of these may represent "at risk" human populations.

9.3.1.4.1.1 Vitamin E deficiency. Rats maintained on vitamin E-deficient diets tended to develop more morphological lesions following exposure to low levels of O_3 than did rats on the usual rations (Plopper et al., 1979; Chow et al., 1981). Rats maintained on a basal vitamin E diet equivalent to the average U. S. human adult intake were exposed to 196 or 392 $\mu g/m^3$ (0.1 or 0.2 ppm) of O_3 24 hr/day for 7 days. According to LM studies, two of the six rats on the basal vitamin E had increased numbers of macrophages in their centriacinar alveoli, a typical response to higher levels of O_3 (Schwartz et al., 1976). Of five rats on the usual rat chow diet exposed to 196 $\mu g/m^3$ (0.1 ppm) O_3 for the same period, LM revealed no increased centriacinar macrophages. LM analysis showed neither dietary group had lesions in the ciliated terminal bronchiolar epithelium. Rats in which lesions were observed by LM had increased macrophages, according to SEM analysis. Analysis by TEM showed that all rats exposed to 196 $\mu g/m^3$ (0.1 ppm) of O_3 differed from controls in that some of the centriacinar type 1 alveolar epithelial cells contained inclusions and were thicker.

Chow et al. (1981) fed month-old rats a basal vitamin E-deficient diet or that diet supplemented with 11 or 110 ppm vitamin E for 38 days, after which they were exposed either to filtered air or to 196 $\mu g/m^3$ (0.1 ppm) of O_3 continuously for 7 days. The morphology of six rats from each diet and exposure group was studied using SEM. None of the filtered-air control animals had lesions. Of the rats exposed to O_3, five of the six on the vitamin E-deficient diet, four of six on the deficient diet supplemented by 11 ppm vitamin E, and one of the six on the deficient diet supplemented by 110 ppm vitamin E developed the typical O_3 lesion as seen with SEM (Schwartz et al., 1976).

Sato et al. (1976a,b, 1978, 1980) exposed vitamin E-deficient and supplemented rats to 588 $\mu g/m^3$ (0.3 ppm) of O_3 3 hr daily for 16 consecutive days or

5 days a week for 7 months. The short-term experiments (Sato et al., 1976a,b) were marred by the presence of chronic respiratory disease in the rats, which may explain the investigators' finding of large amounts of debris and numerous small bodies "so thick that the original surface could not be seen" and their failure to find the typical centriacinar O_3 lesions reported by others (Stephens et al., 1974a; Schwartz et al., 1976). In the latter experiments, Sato et al. (1978, 1980) did not find morphological differences between the vitamin E-depleted and supplemented, filtered-air control rats or between vitamin E-depleted and supplemented, O_3-exposed rats. They did find mild centriacinar O_3 lesions in exposed rats from both vitamin E-deficient and supplemented groups.

Stephens et al. (1983) reported results of exposure of vitamin E-depleted and control young and old rats to 1764 $\mu g/m^3$ (0.9 ppm) of O_3 for 1, 3, 6, 12, 34, 48, and 72 hr. Vitamin E depletion was evaluated by determination of lung tissue levels. Lung response to ozone was based on characteristic tissue nodules previously reported by these authors when using a dissecting microscope rather than on conventional LM, SEM, or TEM. They concluded that response to injury and repair of the lung was independent of the level of vitamin E in lung tissue. Most of these studies included concurrent biochemical evaluations of oxidant metabolism and are discussed in Section 9.3.3.

9.3.1.4.1.2 Age at start of exposure. Although most exposures use young adult experimental animals, there are a few reports of exposures of very young animals (i.e., either before weaning or very soon thereafter).

Bartlett et al. (1974) exposed 3- to 4-week-old rats to 392 $\mu g/m^3$ (0.2 ppm) of O_3 for 30 days. Lung volumes, but not body weights, were significantly greater in the exposed rats. Light microscopy of paraffin sections of conventionally fixed lungs did not reveal differences between exposed and control rats in the lung parenchyma or terminal bronchioles, with the exception of two control animals which had lesions of "typical murine pneumonia." Morphometry was done on thick sections cut by hand with a razor blade from the dorsal and lateral surfaces of air-dried lungs rather than on the paraffin sections of conventionally fixed lungs. Morphometry using these nonrandom samples revealed significantly increased mean alveolar chord lengths and alveolar surface area, but no difference in alveolar numbers.

Freeman et al. (1974) exposed month-old rats to 1058 or 1725 $\mu g/m^3$ (0.54 or 0.88 ppm) of O_3 for periods of 4 hr to 3 weeks. In addition to the centriacinar accumulations of macrophages and hyperplasia of distal airway epithelium seen by others following exposures of young adult animals, they reported an increase in connective tissue elements and collagen-like strands that formed bridges across alveolar openings. Fibrosis was pronounced in the 1725 μg/m^3 (0.88 ppm) group and sometimes extended into terminal bronchioles. Although fixed lung volumes were not determined, the 1725-μg/m^3 (0.88 ppm) group required greater volumes of fixing fluid, evidence of larger lung volumes. In the same research report, Freeman et al. (1974) studied month-old rats exposed to 1764 μg/m^3 (0.9 ppm) of O_3 and 1690 μg/m^3 (0.9 ppm) nitrogen dioxide combined. After 60 days of exposure, they observed the gross and microscopic appearance of advanced experimental emphysema of the type they earlier described following nitrogen dioxide exposure (Freeman et al., 1972). Although others have reported larger fixed lung volumes in exposed young adult rats (Moore and Schwartz, 1981), reports of emphysema following O_3 exposures are uncommon and are discussed in the next subsection of this document.

Stephens et al. (1978) exposed rats ranging in age from 1 to 40 days old to 1666 μg/m^3 (0.85 ppm) O_3 continuously for 24, 48, or 72 hr. Rats exposed to O_3 before weaning at 20 days of age developed little or no evidence of injury, as evaluated by light and electron microscopy. When exposure was initiated after weaning at 20 days of age, centriacinar lesions increased progressively, plateaued at 35 days of age, and continued until approximately 1 year of age.

Barry et al. (1983) exposed 1-day-old male rats and their mother to 490 μg/m^3 (0.25 ppm) of O_3 12 hr/day for 6 weeks. They observed persistence of the centriacinar damage to type 1 epithelial cells and increased centriacinar macrophages. By using LM and TEM morphometry of centriacinar regions, they reported an increase in both type 1 and 2 alveolar epithelial cells. The type 1 cells were smaller in volume, covered less surface, and were thicker. The authors were aware of the above study by Stephens et al. (1978) and discussed the possibility that much of the damage they observed may have occurred in the last 3 weeks of exposure (i.e., after weaning). Changes in lung function evaluated by Raub et al. (1983a) are discussed in Section 9.3.2.

Bils (1970) studied the effects of 1176 to 2548 μg/m^3 (0.6 to 1.3 ppm) of O_3 on mice 4 days old and 1 and 2 months old. From his study, Bils concluded that the endothelium appeared to be the main target of the O_3, a conclusion not supported by more recent studies, which deal mostly with other species. Bils did note the lesions were more severe in the 4-day-old mice than in the 1- or 2-month-old mice.

9.3.1.4.1.3 Effect of pneumonectomy. Two to four weeks following pneumonectomy of rabbits, the contralateral lung increases in volume, weight, collagen, and protein content to approximate that of both lungs from controls, but alveolar multiplication appears dependent on age at surgery. Boatman et al. (1983) exposed pneumonectomized and control rabbits to 784 $\mu g/m^3$ (0.4 ppm) of O_3 7 hr/day, 5 days/week for 6 weeks. They examined the lungs with standard LM and TEM morphometric techniques, but not methods for alveolar numbers. Boatman and co-workers concluded that the lung growth that follows pneumonectomy occurred after O_3 exposure and that no difference existed between males and females in this response.

9.3.1.4.2 Emphysema following ozone exposure. The previous criteria document for O_3 (U.S. Environmental Protection Agency, 1978) cites three published research reports in which emphysema was observed in experimental animals following exposure to $\leq$ 1960 $\mu g/m^3$ ($\leq$ 1 ppm) of O_3 for prolonged periods (P'an et al., 1972; Freeman et al., 1974; Stephens et al., 1976). Since then, no similar exposures (i.e., same species, O_3 concentrations, and times) have been documented to confirm these observations. An additional consideration is the similarity of the centriacinar lesion following O_3 exposure to that seen in young cigarette smokers (Niewoehner et al., 1974; Schwartz et al., 1976; Cosio et al., 1980; Wright et al., 1983; Fujinaka et al., 1985) and the relationship between cigarette smoking and emphysema in humans (U.S. Department of Health, Education, and Welfare, 1967, 1969). Further, animals exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 reportedly have more voluminous lungs than controls (Bartlett et al., 1974; Moore and Schwartz, 1981). Morphometry was used to demonstrate enlarged subpleural alveoli in one of these reports (Bartlett et al., 1974). However, these authors indicate that these subpleural alveoli may not be representative of the whole lung and do not conclude that emphysema was present. Thus, a restudy of these three reports in the 1978 document appears appropriate.

The precise definition of emphysema is critical to reevaluation of these reports. Several professional groups have presented definitions of emphysema (Fletcher et al., 1959; World Health Organization, 1961; American Thoracic Society, 1962). The most recent is the report of a National Heart, Lung and Blood Institute, Division of Lung Diseases Workshop (National Institutes of Health, 1985). In human lungs, "Emphysema is defined as a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis" (National Institutes of Health, 1985). Destruction is further defined: "Destruction in emphysema is defined as non-uniformity in the pattern of respiratory airspace enlargement so that the orderly appearance of the acinus and its components is disturbed and may be lost." The report further indicates "Destruction . . . may be recognized by subgross examination of an inflation-fixed lung slice . . . ." In order to stimulate additional research, the definition of emphysema in animal models was less restrictive. The document states: "An animal model of emphysema is defined as an abnormal state of the lungs in which there is enlargement of the airspaces distal to the terminal bronchiole. Airspace enlargement should be determined qualitatively in appropriate specimens and quantitatively by stereologic methods." Thus in animal models airspace wall destruction need not be present. However, where information from air pollution exposures of animals is to be extrapolated to hazards for humans, the definition of human emphysema must be considered and the presence of airspace wall destruction documented.

Stokinger et al. (1957) reported emphysematous changes in lungs from guinea pigs, rats and hamsters, but not mice or dogs, exposed 6 hr/day, 5 days/week for 14.5 months to a mean concentration of slightly more than 1960 $\mu g/m^3$ (1 ppm) of O_3. With the exception of the dogs, mortality rates were high in both control and exposed animals, ranging in the controls from 25 to 78 percent and in exposed from 11 to 71 percent. The published report indicates that emphysema was present but does not further characterize it as to the presence of only enlarged air spaces (Fletcher et al., 1959) or enlarged air spaces accompanied by destructive changes in alveolar walls (World Health Organization, 1961; American Thoracic Society, 1962). The lungs were fixed via the trachea, making them suitable for studies of experimentally induced emphysema (American Thoracic Society, 1962; National Institutes of Health, 1985). Stokinger et al. (1957) attributed the emphysema in the guinea pigs to the observed bronchial stenosis. Also in the guinea pigs were foci of "extensive linear fibrosis . . . considered to be caused by organization of pneumonic areas." In exposed rats, the mild degree of emphysema "did not exceed the emphysema found in the unexposed control rats." In exposed hamsters, "mild to moderate" emphysema was present, but not in controls. Emphysema is not mentioned in the figure legends, but three of them mention "alveoli are overdistended . . . alveolar spaces are dilated . . . dilation of alveolar ducts and air sacs." Evidence of destruction of alveolar walls is not mentioned. Later, however, Gross et al. (1965), in an unrefereed publication abstracted from a presentation at the seventh Aspen Conference on Research in Emphysema, reviewed the lesions in the hamsters from this exposure and described a "destructive process" that resulted in contraction of interalveolar septa not associated with enlargement of air spaces.

Because the interpretation in this EPA Criteria Document differs from that in the previous document, the details need to be presented. The significance will be discussed in a paragraph at the end of this section. The earlier O_3 criteria document (U.S. Environmental Protection Agency, 1978) cites Stephens et al. (1976), a "long abstract" that appears not to be refereed. This brief article states "rats exposed continuously for long periods (3 - 5 months) to 28,200 $\mu g/m^3$ (15.0 ppm) NO_2 or 1568 $\mu g/m^3$ (0.8 ppm) O_3 develop a disease that closely resembles emphysema" but does not provide additional evidence other than citing five earlier studies by the Stanford group of investigators. Each of those five references was checked for studies of animals exposed to O_3. Three articles describe only NO_2-exposed animals. The fourth reference (Freeman et al., 1973) is to an exposure of dogs to 1960 to 5880 $\mu g/m^3$ (1 to 3 ppm) of O_3 8 to 24 hr daily for 18 months and was cited earlier in this document. Emphysema is not mentioned in that article. Neither is emphysema mentioned in the fifth reference (Stephens et al., 1974b), which was also cited earlier in this document. These investigators did describe (Freeman et al., 1974) a group of month-old rats exposed continuously for 3 weeks to 1725 $\mu g/m^3$ (0.88 ppm) of O_3, half of which died and had "grossly inflated, dry lungs." In this same study, they also exposed month-old rats to a mixture of 1690 $\mu g/m^3$ (0.9 ppm) of NO_2 and O_3 continuously for 60 days, at which time the lungs were grossly enlarged, and "both grossly and microscopically, the appearance of the lungs was characteristic of advanced experimental emphysema" of the type they earlier reported following NO_2 alone at much higher concentrations (Freeman et al., 1972).

The third citation in the O_3 criteria document (U.S. Environmental Protection Agency, 1978) is to P'an et al. (1972). These investigators exposed rabbits to 784 $\mu g/m^3$ (0.4 ppm) of O_3 6 hr/day, 5 days/week for 10 months. Tissues were fixed apparently by immersion rather than infusion via the trachea, which is not in accord with the American Thoracic Society's diagnostic standard for emphysema, making emphysema lesions much more difficult to evaluate accurately. The lesions related to emphysema are only very briefly described and illustrated in only one figure. The authors also report that "all lungs showed some degree of inflammatory infiltrate" and "lungs of the sixth were so congested that visualization of the mural framework of the alveoli was difficult." This is more reaction than reported in other species exposed to this comparatively low O_3 concentration. The rabbits were not specified pathogen-free, nor was the possibility considered that some lesions could be due to infectious agents. Neither did these investigators consider the possibility of spontaneous "emphysema and associated inflammatory changes" which Strawbridge (1960) described in lungs from 155 rabbits of various ages and breeds.

In the studies reviewed in this section, enlargement of air spaces distal to the terminal bronchiole have been described following O_3 exposure of several species of experimental animals. In one study, the enlargement was quantitated using morphometry of air-dried lungs (Bartlett et al., 1974). Destruction of alveolar walls was only briefly described in two reports (P'an et al., 1972; Gross et al., 1965). In one of these studies (P'an et al., 1972) the lungs were apparently fixed by immersion rather than infusion, making a diagnosis of emphysema less reliable (American Thoracic Society, 1962). The other study (Gross et al., 1965) appears to be an unrefereed long abstract rather than a full research report article. Neither of these reports describes lesions which unequivocally meet the criteria for human emphysema as defined by either the American Thoracic Society (1962) the or the National Institutes of Health (1985).

9.3.2 Pulmonary Function Effects

9.3.2.1 Short-Term Exposure. Results of short-term O_3 exposures of experimental animals are shown in Table 9-2. These studies were designed to evaluate the acute changes in lung function associated with O_3 exposure in a variety of species (mice, rats, guinea pigs, sheep, rabbits, cats, monkeys, and dogs) when compared to filtered-air exposure.

The effects of short-term local O_3 exposure of the lung periphery have been examined in dogs by Gertner et al. (1983a,b). A fiber-optic bronchoscope with an outside diameter of 5.5 mm was wedged into a segmental airway and a continuous flow of 196 or 1960 $\mu g/m^3$ (0.1 or 1.0 ppm) of O_3 was flushed through this airway and allowed to escape through the system of collateral channels normally present in the lung periphery. During exposure to either 196 or 1960 $\mu g/m^3$ (0.1 or 1.0 ppm) of O_3, airflow resistance through the collateral channels increased during the first 2 min of exposure. Resistance of the collateral channels continued to increase throughout exposure to 1960 $\mu g/m^3$

TABLE 9-2. EFFECTS OF OZONE ON PULMONARY FUNCTION: SHORT-TERM EXPOSURES

Ozone concentration		Measurement^{a,b} method	Exposure duration & protocol	Observed effects^{c}	Species	Reference
µg/m^3	ppm					
196 1960	0.1 1.0	MAST	30 min	Collateral system resistance increased rapidly during exposure, falling to control levels at 0.1 ppm but continuing to increase at 1.0 ppm of O_3.	Dog	Gertner et al., 1983a,b
431 804 1568	0.22 0.41 0.80	CHEM, NBKI	2 hr	Concentration-dependent increase in f_B for all exposure levels. No change in R_L, TV, or MV. Decreased $C_L$dyn during exposure to 0.4 and 0.8 ppm of O_3.	Guinea pig (200-300 g)	Amdur et al., 1978
470 to 2156	0.24 to 1.1	NBKI	12 hr	Premature airway closure at 6 hr, and 1 and 3 days following exposure, reflected by increased RV, CC, and CV (6 hr and 1 day only). Maximum effect 1 day following exposure, all values returned to normal by 7 days. Distribution of ventilation less uniform 6 hr following exposure. Increased lung distensibility in the mid-range of lung volumes (25-75% TLC) 7 days following exposure.	Rabbit	Inoue et al., 1979
510 980 1960	0.26 0.5 1.0	MAST	2.0 to 6.5 hr	Concentration-dependent increase in R_L during exposure. Decreased C_L and D_LCO but less frequent and less marked than changes in R_L. No change in VC or deflation pressure-volume curves.	Cat	Watanabe et al., 1973
666 1333 2117 2646	0.34 0.68 1.08 1.35	NBKI	2 hr	Increased f_B and decreased TV during exposure to all O_3 concentrations. Increased R_{rs} during exposure to 1.08 and 1.35 ppm of O_3.	Guinea pig (300-400 g)	Murphy et al., 1964
980	0.5	NBKI	2 hr	Slight increase in f_B and R_{rs} (to 113% of control values) during exposure.	Guinea pig (280-540 g)	Yokoyama, 1969

TABLE 9-2. EFFECTS OF OZONE ON PULMONARY FUNCTION: SHORT-TERM EXPOSURES (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration & protocol	Observed effects[c]	Species	Reference
μg/m³	ppm					
1470 1960 3920	0.75 1.0 2.0	CHEM	Continuous 1, 3, 7 or 14 days	The validity of this study is questioned because of low airflow through the exposure chambers and high mortality of exposed animals (66% mortality in rats exposed to 1 ppm of O_3).	Rat	Pepelko et al., 1980
1960	1	NBKI	3 hr	Reduced TLC at air inflation pressure of 30 cm H_2O, 1 to 3 days postexposure but not at 7 days. No difference in lung pressure-volume characteristics during lung inflation with saline.	Rabbit	Yokoyama, 1972, 1973
1960	1	NBKI	6 hr/day, 7 to 8 days	Increased R_L and decreased $C_L dyn$ 1 day following exposure. No change in MEFV curves.	Rabbit	Yokoyama, 1974

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence; NBKI = neutral buffered potassium iodide.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] See Glossary for the definition of pulmonary symbols.

(1.0 ppm) of O_3, but decreased again to control levels during continued exposure to 196 μg/m^3 (0.1 ppm) of O_3. Based on these observations, the authors reported that tolerance appears to develop in the collateral airways to locally delivered O_3 at concentrations of 196 but not 1960 μg/m^3 (0.1 but not 1.0 ppm) of O_3.

Amdur et al. (1978) measured breathing pattern (tidal volume, respiration rate, and minute volume), pulmonary resistance, and dynamic pulmonary compliance in guinea pigs during 2-hr exposures to 431, 804, or 1568 μg/m^3 (0.22, 0.41, or 0.8 ppm) of O_3. Accelerated respiration rates with no significant changes in tidal volume were measured during exposures to all O_3 concentrations. The onset and magnitude of these changes in respiration rate were concentration-dependent, and values of respiration rate remained elevated during a 30-min recovery period following exposure. Pulmonary compliance was significantly lower than pre-exposure values following 1 and 2 hr of exposure to 804 or 1568 μg/m^3 (0.41 or 0.8 ppm) of O_3, and values of compliance remained low during the 30-min recovery period. Changes in dynamic compliance were essentially the same during exposure to either 804 or 1568 μg/m^3 (0.41 or 0.8 ppm) of O_3. These investigators observed no significant change in pulmonary resistance during exposure to O_3. If anything, resistance tended to decrease throughout the exposure and recovery period.

The lack of a significant increase in pulmonary resistance in the Amdur et al. (1978) study is in contrast to the 113 percent increase over pre-exposure values in total respiratory flow resistance measured in guinea pigs exposed to 980 μg/m^3 (0.5 ppm) of O_3 for 2 hr by Yokoyama (1969). Watanabe et al. (1973) also found increased pulmonary flow resistance in cats artificially ventilated through an endotracheal tube with 510, 980, or 1960 μg/m^3 (0.26, 0.50, or 1.00 ppm) of O_3 for 2 to 6.5 hr. Pulmonary resistance had increased to at least 110 percent of control values in all animals after 105 min of exposure to 510 μg/m^3 (0.26 ppm) of O_3, after 63 min of exposure to 980 μg/m^3 (0.50 ppm) of O_3, and after 49 min of exposure to 1960 μg/m^3 (1 ppm) of O_3. Dynamic lung compliance was decreased during O_3 exposure in the Watanabe et al. (1973) study, as it was in the Amdur et al. (1978) study. However, changes in pulmonary compliance measured by Watanabe et al. (1973) occurred less frequently and were less severe (based on percentage changes from pre-exposure control values) than changes in pulmonary resistance.

Like Amdur et al. (1978) and Yokoyama (1969), Murphy et al. (1964) also measured breathing pattern and respiratory flow resistance in guinea pigs during 2-hr O_3 exposures. These investigators found concentration-related increases in respiration rate during exposure to 666, 1333, 2117 or 2646 µg/m^3 (0.34, 0.68, 1.08, or 1.35 ppm) of O_3. Respiratory flow resistance was increased (to 148 and 170 percent of pre-exposure values) in guinea pigs exposed to 2117 and 2646 µg/m^3 (1.08 and 1.35 ppm) of O_3 respectively. Pulmonary compliance was not measured.

The variability in measurements of pulmonary resistance following O_3 exposure can be attributed to a number of factors including the following: frequency characteristics of the monitoring equipment and measurement techniques utilized, the influence of anesthetics, and the intraspecies differences in airway reactivity of guinea pigs. The latter point was the subject of critical review in the assessment of toxicological effects from particulate matter and sulfur oxides (U.S. Environmental Protection Agency, 1982).

Recovery of guinea pigs from short-term O_3 exposure was substantially different in the above three studies. Animals exposed by Amdur et al. (1978) showed little or no return toward pre-exposure values for any of the measured parameters during a 30-min recovery period following exposure. In guinea pigs exposed by Murphy et al. (1964) and Yokoyama (1969), respiration rates had returned almost to pre-exposure values by 30 min following exposure. The development of more persistent lung-function changes following O_3 exposure in the Amdur et al. study (1978) may be attributed to the small size and associated immaturity of these guinea pigs (200 to 300 g) compared with those in the studies by Murphy et al. (1964) (300 to 400 g) and Yokoyama (1969) (280 to 540 g). In an earlier study, Amdur et al. (1952) showed that young guinea pigs 1 to 2 months old were significantly more sensitive to inhaled sulfuric acid aerosols than 12- to 18-month-old animals. The use of ether anesthesia and placement of an intrapleural catheter by Amdur et al. (1978) but not by Murphy et al. (1964) or Yokoyama (1969) may also have sensitized the animals exposed by Amdur et al. (1978) to effects of O_3.

Inoue et al. (1979) exposed rabbits to 470 to 2156 µg/m^3 (0.24 to 1.1 ppm) of O_3 for 12 hr and performed lung function tests 6 hr, and 1, 3, and 7 days following exposure. These rabbits showed functional evidence of premature airway closure with increased trapped gas at low lung volumes 6 hr, 1 day, and 3 days following exposure. Functional changes indicating premature airway closure included increased values of closing capacity, residual volume, and closing volume. Lung quasistatic pressure-volume measurements showed higher lung volumes at lung distending pressures from 0 to -10 cm of H_2O. These lung function changes were greatest 1 day following exposure and had disappeared by 7 days following exposure. Distribution of ventilation in the lung was less uniform in O_3-exposed animals only at 6 hr following exposure. By 7 days following the initial 12-hr O_3 exposure, the only significant functional change was an increased lung distensibility in the midrange of lung volumes (from 25 to 75 percent total lung capacity).

Earlier studies by Yokoyama (1972), in which rabbits were exposed to 1 ppm of O_3 for 3 hr, showed a timing of lung function changes similar to that observed by Inoue et al. (1979). For both studies, maximum changes in O_3-exposed animals were observed 1 day following exposure and had disappeared by 7 to 14 days following exposure. However, in some aspects, the Yokoyama (1972) study was substantially different from that of Inoue et al. (1979). Yokoyama (1972) found reduced maximum lung volume at an air inflation pressure of 30 cm of H_2O, whereas Inoue et al. (1979) found no difference in maximum lung volume. Yokoyama (1972) does not show lung pressure-volume curves at pressures less than atmospheric pressure, so premature airway closure and gas trapping cannot be evaluated in this study. One factor that may contribute to differences between these two studies is the use of an excised lung preparation by Yokoyama (1972) compared with evaluation of intact lungs in anesthetized rabbits by Inoue et al. (1979).

Yokoyama (1974) also evaluated lung function in rabbits following exposure to 1960 $\mu g/m^3$ (1 ppm) of O_3, 6 hr/day, for 7 to 8 days. He found increased pulmonary resistance and decreased dynamic compliance in O_3-exposed animals compared to air-exposed control animals. Static pressure-volume curves and maximum expiratory flow-volume curves were not significantly different between the two groups.

9.3.2.2 Long-Term Exposure. Table 9-3 summarizes results of long-term O_3 exposures. Raub et al. (1983a) exposed neonatal and young adult (6-week-old) rats to 157, 235, or 490 $\mu g/m^3$ (0.08, 0.12, or 0.25 ppm) of O_3 12 hr/day, 7 days/week for 6 weeks. Lung function changes were observed primarily in neonatal rats following 6 weeks of O_3 exposure. Peak inspiratory flow measured in these animals during spontaneous respiration was significantly lower following exposure to 235 or 490 $\mu g/m^3$ (0.12 or 0.25 ppm) of O_3. Lung volumes measured at high distending pressures were significantly higher in neonatal animals exposed to 490 $\mu g/m^3$ (0.25 ppm) of O_3 for 6 weeks than in control animals. These results are consistent with increased lung volumes measured during lung inflation with either air or saline by Bartlett et al. (1974)

TABLE 9-3. EFFECTS OF OZONE ON PULMONARY FUNCTION: LONG-TERM EXPOSURES

Ozone concentration		Measurement^{a,b} method	Exposure duration & protocol	Observed effects^{c}	Species	Reference
$\mu g/m^3$	ppm					
157 235 490	0.08 0.12 0.25	CHEM	6 weeks, 12 hr/day, 7 days/week	Increased end expiratory lung volume in adult rats and increased lung volumes at high distending pressures in neonatal rats exposed to 0.25 ppm of O_3. Reduced peak inspiratory flow in neonatal rats exposed to 0.12 or 0.25 ppm of O_3.	Rat (neonate or 6-week-old young adult)	Raub et al., 1983a
392	0.2	MAST, NBKI	28 to 32 days, continuous	Increased lung distensibility in O_3-exposed rats at high lung volumes (95-100% TLC) during inflation with air or saline.	Rat (3 to 4 weeks)	Bartlett et al., 1974
392 1568	0.2 0.8	UV or CHEM NBKI	62 exposures, 6 hr/day, 5 days/week	Increased R_L (not related to concentration) in rats exposed to 0.2 or 0.8 ppm of O_3. Lung volumes at high distending pressures (VC and TLC) were increased at 0.8 ppm and FEF_{25} and FEF_{10} were decreased at 0.2 and 0.8 ppm of O_3.	Rat (10 weeks)	Costa et al., 1983
784	0.4	NBKI	6 weeks, 7 hr/day, 5 days/week	Increased alveolar wall extensibility at yield and break points, increased hysteresis ratio, and decreased stress at moderate extensions. Fixed lung volume increased 15%. Lung growth following pneumonectomy prevented these changes to O_3 exposure.	Rabbit	Martin et al., 1983
882	0.45	MAST	6 to 7 weeks, 6 hr/day, 6 days/week	No effect of exposure on lung pressure-volume curves.	Rat	Yokoyama and Ichikawa, 1974
980 1568	0.5 0.8	UV, NBKI	7, 28, or 90 days; 8 hr/day	Decreased quasistatic compliance (not related to concentration).	Monkey (Bonnet)	Eustis et al., 1981

TABLE 9-3. EFFECTS OF OZONE ON PULMONARY FUNCTION: LONG-TERM EXPOSURES (continued)

Ozone concentration µg/m^3	Ozone concentration ppm	Measurement[a,b] method	Exposure duration & protocol	Observed effects[c]	Species	Reference
980 1960	0.5 1.0	MAST, NBKI	3 and 6 hr/day for up to 60 days	Increased resistance of central airways after 3-hr daily exposures to 1.0 ppm for 30 days; increased resistance of peripheral airways after 6-hr daily exposures to 0.5 ppm O_3 for 60 days.	Rat	Yokoyama et al., 1984
1254	0.64	UV, NBKI	7 or 20 days	Increased peripheral resistance in rats exposed for 7 days but not 20 days; decreased lung reactance at high frequencies in both groups.	Rat	Kotlikoff et al., 1984
1254	0.64	UV, NBKI	1 year, 8 hr/day, 7 days/week	Following 6 months of exposure, ventilation was less homogeneous and R_L was increased. Following 12 months of exposure, R_L remained elevated and forced expiratory maneuvers showed small airway dysfunction (decreased FEV_1 and $FEF_{12\cdot 5}$). During the 3-month recovery period following exposure, C_Lst decreased.	Monkey (Bonnet)	Wegner, 1982

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence; UV = UV photometry.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] See Glossary for the definition of pulmonary symbols.

following exposure of young rats (3- to 4-week-old) to 392 $\mu g/m^3$ (0.2 ppm) of O_3 continuously for 28 to 32 days. Moore and Schwartz (1981) also found an increased fixed lung volume (following lung perfusion at 30 cm inflation pressure with Karnovsky's fixative) after 180 days of continuous exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3. Yokoyama and Ichikawa (1974) found no change in lung static pressure-volume curves in mature rats exposed to 882 $\mu g/m^3$ (0.45 ppm) of O_3 6 hr/day, 6 days/ week for 6 to 7 weeks.

Martin et al. (1983) studied the mechanical properties of the alveolar wall from rabbits exposed to 784 $\mu g/m^3$ (0.4 ppm) of O_3, 7 hr/day, 5 days/week for 6 weeks. A marked increase in the maximum extensibility of the alveolar wall and a greater energy loss with length-tension cycling (hysteresis) were found following exposure. A 15-percent increase in fixed lung volume following perfusion at 20 cm of H_2O was also reported following O_3 exposure, which is similar to the fixed lung volume changes reported by Moore and Schwartz (1981). Morphology and morphometry of paired lungs or lungs from animals similarly exposed to O_3 is reported in Section 9.3.1.4.

Costa et al. (1983) evaluated lung function changes in rats exposed to 392, 1568, or 3920 $\mu g/m^3$ (0.2, 0.8, or 2 ppm) of O_3, 6 hr/day, 5 days/week for 62 exposure days. (This report will not discuss effects in animals exposed to 2 ppm of O_3). These investigators found increased pulmonary resistance (not concentration-related) in rats exposed to 392 or 1568 $\mu g/m^3$ (0.2 or 0.8 ppm) of O_3. Lung volumes measured at high distending pressure (VC and TLC) were increased following exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3. Similar changes in lung distensibility were observed by Raub et al. (1983a), Bartlett et al. (1974), Moore and Schwartz (1981), and Martin et al. (1983). Costa et al. (1983) also observed decreased (not concentration-related) maximum expiratory flows at low lung volumes (25 and 10 percent of VC) in rats exposed to 392 or 1568 $\mu g/m^3$ (0.2 or 0.8 ppm) of O_3. Changes in maximum flow at low lung volumes indicate peripheral airway dysfunction and may be related to reduced parenchymal elasticity or narrowing of the airway lumen.

Yokoyama et al. (1984) evaluated lung function in 7-week-old rats immediately after exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3, 3 hr/day for 14 and 30 consecutive days and in rats of two different ages (4- and 10-week-old) one day after exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3, 6 hr/day for 60 consecutive days. Pulmonary flow resistance (R_L) increased after O_3 exposure; however, the pattern of this change was different between both types of exposure. Increased R_L occurred over a wide range of lung deflation pressures in the former exposure while R_L increased only at lower pressures after the latter exposure. The authors interpreted these changes as an indication of increased central airway resistance in rats exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 for 30 days and increased peripheral airway resistance in rats exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3 for 60 days. These changes were also consistent with morphological findings of greater mucous secretions in large bronchi of the animals exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 and in the peripheral airways of animals exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3. No changes in static deflation volume-pressure curves of the lungs were found after either exposure nor were there any differences in effects that could be attributed to the age of rats at the start of exposure.

Eustis et al. (1981) evaluated lung function in bonnet monkeys (_Macaca radiata_) exposed to 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3, 8 hr/day for 7, 28, or 90 days. This study appeared to be preliminary (range-finding) for the long-term study reported by Wegner (1982). Only a limited number of animals were evaluated at each time point (1 per exposure group at 7 days, 2 at 28 days, and 3 at 90 days). With so few animals tested and tests made following three different exposure periods, little significant lung-function data related to O_3 exposure were generated. When pooling results from all exposure times and O_3 concentrations, quasi-static lung compliance was significantly different in O_3-exposed animals than in control animals. Compliance tended to decrease from pre-exposure values in control animals and increase in O_3-exposed animals.

Wegner (1982) evaluated lung function in 32 bonnet monkeys, 16 of which were exposed to 1254 $\mu g/m^3$ (0.64 ppm) of O_3, 8 hr/day, 7 days/week for 1 year. Lung function tests were performed pre-exposure, following 6 and 12 months of exposure, and following a 3-month postexposure recovery period. In addition to measurements of carbon monoxide diffusion capacity of the lungs (D_{LCO}), lung volumes, quasi-static pulmonary compliance ($C_{st(L)}$) and partial and maximum expiratory flow-volume curves by standard techniques, frequency dependence of compliance and resistance and pulmonary impedance from 2-32Hz were measured by a forced oscillation technique. The addition of these latter measurements may elucidate more clearly than ever before the site and nature of lung impairment caused by exposure to toxic compounds.

Following six months of O_3 exposure, pulmonary resistance and frequency dependence of pulmonary compliance were significantly increased. After 12 months, the O_3 exposure had significantly increased pulmonary resistance and inertance (related to the pressure required to accelerate air and lung tissue), and forced expiratory maneuvers showed decreased flows at low lung volumes (12.5 percent VC) and decreased volume expired in 1 sec (FEV_1). Wegner (1982) suggested that because lung volumes and pulmonary compliance were not affected in O_3-exposed animals, changes in forced expiratory function were more likely caused by narrowing of the peripheral airways than by decreased small airway stiffness. Rigid analysis of the pulmonary impedance data by linear-lumped-parameter modeling suggested that the increase in pulmonary resistance was due to central as well as peripheral airway narrowing.

During the 3-month recovery period following exposure, static lung compliance tended to decrease in both O_3-exposed and control animals. However, the decrease in compliance was significantly greater in O_3-exposed animals than in control animals. No other significant differences were measured following the 3-month recovery period, although values for O_3-exposed animals remained substantially different from those for control animals, suggesting that full recovery was not complete.

The forced oscillation technique has also been utilized in rats exposed to 1254 $\mu g/m^3$ (0.64 ppm) of O_3 for either 7 or 20 days (Kotlikoff et al., 1984). In an attempt to further characterize O_3-induced changes in central and peripheral distribution of mechanical properties of the respiratory system, impedance spectra of O_3-exposed rats were compared to the spectra of normal rats. The effective resistance was higher at all frequencies in the 7-day exposed rats but no consistent differences were observed by 20 days. The effective reactance, however, was significantly lower than control in both the 7- and 20-day exposed rats. These changes in respiratory system impedance demonstrate evidence of mechanical alterations in the peripheral airways of rats for as long as 20 days of O_3 exposure.

9.3.2.3 Airway Reactivity. Ozone potentiates the effects of drugs that constrict airway smooth muscle in mice, guinea pigs, dogs, sheep, and humans (Table 9-4). Early experimental evidence for hyperreactivity to bronchoconstrictive drugs following O_3 exposure was provided by Easton and Murphy (1967). Although much of their work was done with very high O_3 concentrations (9800 to 11760 $\mu g/m^3$, 5 to 6 ppm), they did show that mortality from a single subcutaneous injection of histamine was higher in guinea pigs exposed to 980 or 1960 $\mu g/m^3$ (0.5 or 1 ppm) of O_3 for 2 hr (33 and 50 percent mortality, respectively) compared with the mortality of air-exposed control animals. The animals appeared to die from massive bronchoconstriction, with the lungs remaining fully inflated instead of collapsing when the chest was opened.

TABLE 9-4. EFFECTS OF OZONE ON PULMONARY FUNCTION: AIRWAY REACTIVITY

Ozone concentration		Measurement[a] method	Exposure duration & protocol	Observed effects[b]	Species	Reference
$\mu g/m^3$	ppm					
196 to 1568	0.1 to 0.8	CHEM	1 hr	Subcutaneous injection of histamine 2 hr following O_3 exposure caused a greater increase in R_L following exposure to 0.8 ppm of O_3 and a greater decrease in $C_L$dyn following exposure to all O_3 concentrations (magnitude of C_L changes not related to O_3 concentration).	Guinea pig (200-300 g)	Gordon and Amdur, 1980
196 1568	0.1 0.8	CHEM	1 hr	Decreased diaphragm and lung cholinesterase activity; parathion-treated animals had increased peak airway resistance compared to controls, but the difference was not statistically significant.	Guinea pig	Gordon et al., 1981
196 1960	0.1 1.0	MAST	10-30 min	Bilateral vagotomy: completely blocked increased peripheral lung resistance from 0.1 ppm of O_3 but not histamine; only partially blocked response from 1.0 ppm of O_3. Histamine-induced airway reactivity increased during 1.0 ppm but not 0.1 ppm of O_3 exposure and was not blocked by atropine or vagotomy.	Dog	Gertner et al., 1983a,b,c, 1984 Kaplan et al., 1981
980	0.5	CHEM	2 hr	Increased number of mast cells and lymphocytes in tracheal lavage 24 hr after exposure.	Sheep	Sielczak et al., 1983
980 1568	0.5 0.8	MAST	Continuous, 13 to 16 days of O_3 exposure in four periods (3 to 5 days each) separated by 3 to 8 days of breathing air	Repeated exposures to 0.5 or 0.8 ppm of O_3 plus aerosolized ovalbumin resulted in greater mortality from anaphylactic shock produced by intravenous injection of ovalbumin compared with effects of ovalbumin injection in mice repeatedly exposed to ovalbumin aerosols but no O_3.	Mouse	Osebold et al., 1980
980 2156	0.5 1.1	NBKI	2 hr	Increased histamine-induced mortality immediately following exposure to 0.5 or 1.1 ppm of O_3.	Guinea pig	Easton and Murphy, 1967
980 1960 3920	0.5 1 2	CHEM	2 hr	Increased airway reactivity to aerosolized carbachol 24 hr but not immediately following exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3 with no change in R_L, FRC, C_Lst, or tracheal mucous velocity. Increased R_L 24 hr following exposure and airway reactivity immediately and 24 hr following exposure (1 ppm).	Sheep	Abraham et al., 1980

TABLE 9-4. EFFECTS OF OZONE ON PULMONARY FUNCTION: AIRWAY REACTIVITY (continued)

Ozone concentration		Measurement[a] method	Exposure duration & protocol	Observed effects[b]	Species	Reference
µg/m³	ppm					
980	0.5	CHEM	2 hr/day for 2 days	No effect on airway responsss to inhaled carbachol 1 day after O_3 exposure; airway reactivity increased 34% and airway sensitivity increased 31% with intravenous carbachol challenge.	Sheep	Abraham et al., 1984a
980 1960	0.5 1.0	CHEM	2 hr	Airway responsiveness and airway permeability to histamine increased 1 day after exposure to 0.5 ppm O_3 (n=6) and in 4/7 exposed to 1.0 ppm O_3; directional changes in airway responsiveness paralleled directional changes in airway permeability.	Sheep	Abraham et al., 1984b
1100 to 1666	0.56 to 0.85	CHEM	2 hr	Abnormal, rapid, shallow breathing in conscious dogs while walking on a treadmill following O_3 exposures. Maximal 1- to 3-hr postexposure, normal 24-hour postexposure. Abnormal breathing not affected by drug-induced bronchodilatation (inhaled isoproteronol) but abolished by vagal cooling. Increased respiration rate caused by inhalation of aerosolized histamine after O_3 exposure also blocked by vagal cooling but not by isoproteronol.	Dog	Lee et al., 1979
1313	0.67	CHEM	2 hr	Abnormal, rapid, shallow breathing during exposure to air containing low O_2 or high CO_2 immediately following O_3 exposure. Abnormal breathing not affected by inhaled atropine aerosols or inhaled isoproteronol aerosols but abolished by vagal cooling.	Dog	Lee et al., 1980
1372 to 2352	0.7 to 1.2	CHEM	2 hr	Greater increase in R_L caused by histamine aerosol inhalation 24 hr following O_3 exposure. No hyperreactivity to histamine 1 hr following O_3 exposure. Drug-induced bronchodilatation (inhaled isoproteronol) blocked any increase in R_L before or after O_3 exposure. Inhalation of atropine or vagal cooling (to block reflex bronchoconstriction) prevented O_3-induced reactivity to histamine.	Dog	Lee et al., 1977

TABLE 9-4. EFFECTS OF OZONE ON PULMONARY FUNCTION: AIRWAY REACTIVITY (continued)

Ozone concentration		Measurement[a] method	Exposure duration & protocol	Observed effects[b]	Species	Reference
μg/m³	ppm					
1960 2352	1.0 1.2	CHEM	1 hr	R_L increased and C_L decreased with subcutaneous histamine 2 hr after exposure; responsiveness was not blocked by atropine or vagotomy. No change in static compliance after subcutaneous histamine injection.	Guinea pig	Gordon et al., 1984
1960 4312 5880	1.0 2.2 3.0	UV	2 hr	Marked increase in airway responsiveness to inhaled ACh and histamine 1 hr after exposure; increased to a lesser degree 1 day later, and returned to control levels by 1 week. Effects possibly linked to acute inflammatory response.	Dog	Holtzman et al., 1983a,b Fabbri et al., 1984
5880	3.0	UV	2 hr	Airway responsiveness to inhaled ACh was prevented by indomethacin pretreatmen not the airway infiltration by neutrophils. Both responsiveness and neutrophil infiltration were prevented by hydroxyurea pretreatment.	Dog	O'Byrne et al., 1984a,b
5880	3.0	UV	2 hr	SR_{aw} measured with intravenous ACh and/or inhaled ACh or methacholine increased similarly 14 hr after after exposure; airway reactivity to inhaled bronchoconstrictors returned to baseline levels 2 days after exposure while responses to intravenous ACh persisted.	Guinea pig	Roum and Murlas, 1984
5880	3.0	UV	2 hr	SR_{aw} increased with intravenous ACh; maximal response 2 hr after exposure; remission by the 4th day. Airway infiltration of neutrophils occurred later and lasted longer than airway reactivity.	Guinea pig	Murlas and Roum (1985)

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence; NBKI = neutral buffered potassium iodide.

[b] See Glossary for the definition of pulmonary symbols.

Abraham et al. (1980) evaluated airway reactivity in sheep from measurements of pulmonary resistance following inhaled carbachol aerosols. Carbachol causes bronchoconstriction by stimulating airway smooth muscle at receptor sites that are normally stimulated by release of acetylcholine from terminals of the vagus nerve. Pulmonary resistance during inhalation of carbachol aerosols was significantly higher than pre-exposure values at 24 hr postexposure but not immediately following a 2-hr exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3. This O_3 exposure did not affect resting end-expiratory lung volume (functional residual capacity) or static lung compliance. In sheep exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 for 2 hr, baseline resistance (before carbachol aerosol inhalation) was elevated 24 hr following exposure, and airway reactivity to carbachol was increased immediately and 24 hr following O_3 exposure.

To determine if O_3-induced airway secretions limit the penetration of an inhaled bronchoconstrictor, Abraham et al. (1984a) compared airway responsiveness to inhaled and intravenous carbachol before and 24 hours after exposure to O_3. Adult female sheep were exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3, 2 hr/day for 2 consecutive days. Airway sensitivity was defined as the largest increase in specific lung resistance after carbachol challenge and airway reactivity as the slope of the dose-response curve. There were no significant differences between pre- and postexposure responses to inhalation challenge. However, O_3 exposure increased mean airway reactivity and sensitivity by 34 and 31 percent, respectively, using intravenous challenge. Since carbachol causes direct stimulation of airway smooth muscle, the authors suggested that O_3 may have decreased penetration of the inhaled carbachol to the airway smooth muscle as the result of increased airway secretion. This hypothesis is supported by a previous study showing that changes in airway responsiveness to inhaled histamine following exposure to O_3 may have been related to changes in airway permeability to histamine (Abraham et al., 1984b).

A study in awake guinea pigs comparing changes in airway reactivity with intravenous and inhaled bronchoconstrictors after exposure to a high concentration of O_3 has suggested that mechanisms other than increased airway permeability may be involved. Roum and Murlas (1984) measured changes in specific airway resistance with intravenous and/or inhaled acetylcholine or methacholine from 4 hr to 2 days after exposure to 5880 $\mu g/m^3$ (3.0 ppm) of O_3 for 2 hr. Airway hyperreactivity by either route was similar within 24 hr of exposure. Two days after exposure airway reactivity to bronchoconstrictor inhalation returned to baseline levels while responses to intravenous acetylcholine persisted. The consistent early changes in airway reactivity after O_3 exposure with either intravenous or inhaled bronchoconstrictors indicated that this response may be independent of the route of delivery. However, it is also possible that there may be more than one mechanism responsible for O_3-induced airway hyperreactivity depending on the concentration of O_3 reaching the airway tissue and on interspecies differences in cellular responsiveness to O_3.

Gordon and Amdur (1980) evaluated airway reactivity to subcutaneously injected histamine in awake guinea pigs following a 1-hr exposure to 196 to 1568 $\mu g/m^3$ (0.1 to 0.8 ppm) of O_3. Airway reactivity to histamine was maximal 2 to 6 hr following O_3 exposure and returned to control levels by 24 hr following exposure. The histamine-induced increase in pulmonary resistance was greater in guinea pigs exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 than in air-exposed control animals. Pulmonary compliance decreased more following histamine injection in all O_3-exposed groups than in air-exposed controls, but there were no differences in the histamine-induced decreases in pulmonary compliance between any of the O_3 concentrations (from 196 to 1568 $\mu g/m^3$; 0.1 to 0.8 ppm).

Gordon et al. (1981) studied the effect of O_3 on tissue cholinesterases to see if they were responsible for the bronchial reactivity observed following challenges with bronchoconstrictor analogs of acetylcholine. Guinea pigs were exposed to clean air or 196 or 1568 $\mu g/m^3$ (0.1 or 0.8 ppm) of O_3 for 1 hr. After 2 hr, brain, lung, and diaphragm samples were analyzed for cholinesterase activity. Brain cholinesterase activity was not affected, but lung cholinesterase underwent a 17 percent decrease in activity at 196 $\mu g/m^3$ (0.1 ppm) and a 16 percent decrease at 1568 $\mu g/m^3$ (0.8 ppm). Ozone at 1568 $\mu g/m^3$ (0.8 ppm) also decreased the diaphragm cholinesterase activity by 14 percent. To provide long-term inhibition of cholinesterase, guinea pigs were treated with parathion, an irreversible cholinesterase inhibitor. Airway resistance tended to increase following histamine challenge in the parathion-treated guinea pigs, but the difference was not statistically significant because of large variations in response. The authors suggested that cholinesterase inhibition by O_3 may contribute to the O_3-induced bronchial reactivity, as already reported. Presumably, the decreased cholinesterase activity could result in higher acetylcholine concentrations in the bronchial muscle. A cholinergic-related stimulus, such as occurs with O_3 exposure, should then increase the contraction of the bronchus. The persistence of this activity is not known.

Gordon et al. (1984) extended these studies to determine the site of the airway hyperresponsiveness to histamine-induced airway constriction after O_3 exposure. Anesthetized guinea pigs were evaluated for response to subcutaneous histamine 2 hr after exposure to 1960 or 2352 $\mu g/m^3$ (1.0 or 1.2 ppm) of O_3 for 1 hr. Respiratory resistance increased and dynamic compliance decreased in O_3-exposed animals, as previously reported (Gordon and Amdur, 1980). However, static compliance changes after histamine were similar in the air- and O_3-exposed animals, suggesting that the site of hyperresponsiveness was in the conducting airways rather than the parenchyma. In addition, enhanced airway responsiveness to histamine was not blocked by atropine or vagotomy, indicating a minimal level of vagal involvement. The significantly greater increase in respiratory resistance caused by efferent electrical stimulation of the vagus in O_3-exposed animals suggested that other mechanisms, such as changes in the airway smooth muscle, may be responsible for the hyperexcitability following O_3 exposure. However, in vitro studies on isolated parenchymal strips removed from the lungs of air- and O_3-exposed animals failed to show any differences in the contractile responses to histamine or carbachol.

Lee et al. (1977) evaluated airway reactivity in O_3-exposed dogs from changes in pulmonary resistance induced by histamine aerosol inhalation. Dogs were exposed to 1372 to 2352 $\mu g/m^3$ (0.7 to 1.2 ppm) of O_3 for 2 hr. Airway reactivity to inhaled histamine aerosols was significantly greater 24 hr but not 1 hr after O_3 exposure. Bronchodilatation induced by inhalation of isoproterenol aerosols prevented any change in resistance following histamine exposure. This experiment showed that the increased resistance normally observed following histamine exposure was caused by constriction of airway smooth muscle and not by edema or increased mucous production, which would not be prevented by isoproterenol bronchodilatation. Administration of atropine (which blocks bronchoconstrictor activity coming from the vagus nerve) or vagal cooling (which blocks both sensory receptor activity traveling from the lung to the brain and bronchoconstrictor activity going from the brain to the lung) decreased the response to histamine both before and following O_3 exposure and abolished the hyperreactive airway response. These experiments showed that the increased sensitivity to histamine following O_3 exposure was caused by heightened activity of vagal bronchoconstrictor reflexes.

Although the work of Lee et al. (1977) provides evidence that stimulation of vagal reflexes by histamine is in part responsible for the increased airway reactivity found in dogs following O_3 exposure, Kaplan et al. (1981) found that local responses to histamine in the lung periphery may not be mediated by a significant vagal component. When monodispersed histamine aerosols were delivered to separate sublobar bronchi in dogs through a 5.5 mm diameter fiber-optic bronchoscope, collateral airflow resistance increased both before and after bilateral cervical vagotomy. In follow-up studies that used similar techniques, Gertner et al. (1983a,b,c; 1984) described the role of vagal reflexes in the response of the lung periphery to locally administered histamine and O_3. Collateral resistance increased during separate 30-min exposures to either 196 $\mu g/m^3$ (0.1 ppm) of O_3 or 1.5 $\times 10^{-6}$ mg/m^3 of histamine. However, although parasympathetic blockade (atropine or bilateral cervical vagotomy) prevented the responses to O_3, it did not prevent the responses to histamine (Gertner et al., 1983b). To determine if O_3-induced increases in collateral resistance in the lung periphery were dependent on vagal reflexes, aerosolized neostigmine was administered locally to maintain parasympathetic tone. Responses to 196 $\mu g/m^3$ (0.1 ppm) of O_3 in the lung periphery were enhanced only if the vagi were intact and were limited to the challenged region (Gertner et al., 1984). When larger areas were exposed, vagally mediated responses occurred in both lungs. In addition, the characteristics of responses to high concentrations of O_3 differ markedly from responses to the lower concentrations. A 30-min exposure to 196 $\mu g/m^3$ (0.1 ppm) of O_3 did not affect the airway responsiveness to histamine, but when the O_3 exposure was increased to 1960 $\mu g/m^3$ (1.0 ppm) for 10 min, histamine produced greater increases in collateral resistance that were not abolished by parasympathetic blockade (Gertner et al., 1983c). Exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 for 30 min produced an increase in collateral resistance that was mediated by the parasympathetic system in the early phase of the response and related in part to histamine release in the late phase of the response (Gertner et al., 1983a). Results from this series of studies by Kaplan et al. (1981) and Gertner et al. (1983a,b,c; 1984) are difficult to interpret because of the small numbers of animals in each test group and large variations in response. In addition, because peripheral resistance contributes only a small part to total pulmonary resistance, the findings of these authors do not necessarily contradict the work of Lee et al. (1977). Rather, all the studies taken together suggest that the periphery of the lung may respond differently from the larger conducting airways during exposure to O_3 and that factors in addition to vagal bronchoconstrictor reflexes can produce an increased airway reactivity to histamine.

Holtzman et al. (1983a) reported the time course of O_3-induced airway hyperreactivity in dogs exposed to 1960 and 4312 $\mu g/m^3$ (1.0 and 2.2 ppm) of O_3 for 2 hrs. Airway responsiveness to acetylcholine in 7 dogs increased markedly 1 hr, and to a lesser extent, 24 hr after exposure to 4312 $\mu g/m^3$ (2.2 ppm) of O_3, returning to control levels by 1 week after exposure. Ozone-induced increases in airway responsiveness to histamine were similar following exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3, but data were reported for only 2 dogs. The authors suggested that the time course of the O_3 effect may be linked to acute airway inflammation. In a coincident publication, Holtzman et al. (1983b) found a strong association between airway hyperreactivity and tracheal inflammation in dogs 1 hr following a 2-hr exposure to 4116 $\mu g/m^3$ (2.1 ppm) of O_3. Airway reactivity was assessed from the increase in pulmonary resistance following inhalation of acetylcholine aerosols, and airway reactivity was increased in 6 of 10 O_3-exposed dogs. The number of neutrophils present in a tracheal biopsy, a measure of inflammation, was increased only in the 6 dogs that were hyperreactive to acetylcholine. These observations have recently been extended to show an association of O_3-induced increases in airway responsiveness with inflammation in more distal airways (Fabbri et al., 1984). The number of neutrophils as well as ciliated epithelial cells in fluid recovered from bronchoalveolar lavage was increased in 5 dogs that were hyperreactive to acetylcholine following a 2-hr exposure to 5880 $\mu g/m^3$ (3.0 ppm) of O_3 without significant changes in the numbers of macrophages, lymphocytes, or eosinophils. In dogs depleted of neutrophils by treatment with hydroxyurea, O_3 exposure to 5880 $\mu g/m^3$ (3.0 ppm) of O_3 for 2 hr caused the desquamation of epithelial cells but airway responsiveness to inhaled acetylcholine was prevented (O'Byrne et al., 1984a). This observation suggests that O_3-induced hyperresponsiveness may depend on the mobilization of neutrophils into the airways.

The authors have expanded their work to speculate that the neutrophils produce mediators that are responsible for the increased responsiveness of airways (O'Byrne et al., 1984b). In dogs pretreated with indomethacin, a prostaglandin synthetase inhibitor, exposure to 5880 $\mu g/m^3$ (3.0 ppm) of O_3 for 2 hr had no effect on airway responsiveness to inhaled acetylcholine but there was a significant increase in the number of neutrophils in the airway epithelium. While these results suggest that oxidation products of arachidonic acid, possibly prostaglandins or thromboxane (O'Byrne et al., 1984c), may be released by the neutrophils, they are not conclusive. Therefore, the identity of the specific inflammatory cells or of the responsible mediators is still uncertain.

The results of tracheal lavage in sheep exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3 for 2 hr suggest that the migration of mast cells into the airways may also have important implications for reactive airways and allergic airway disease (Sielczak et al., 1983). Nasotracheal-tube exposure to O_3 in 7 sheep resulted in an increased number of mast cells and lymphocytes 24 hr after exposure, suggesting an association between an enhanced inflammatory response and O_3-induced bronchial reactivity reported previously in sheep (Abraham et al., 1980).

Additional evidence in guinea pigs suggests that O_3-induced bronchial hyperreactivity may be due to airway mucosal injury and mast cell infiltration (Murlas and Roum, 1985). Specific airway resistance was measured as a function of increasing intravenous acetylcholine doses for varying periods of from 2 hr up to 4 days after exposure to 5880 $\mu g/m^3$ (3.0 ppm) of O_3 for 2 hr. The largest airway response to acetylcholine challenge occurred 2 hr after O_3 exposure with complete remission by the fourth day. Neutrophil infiltration occurred later and lasted longer despite the remission in airway hyperreactivity suggesting that the influx of neutrophils was a result of the initial damage and not a direct cause of increased airway responsivensss.

Increased drug-induced bronchoconstriction is not the only indicator of airway hyperreactivity following O_3 exposure. Animal experiments were designed to investigate the mechanisms responsible for the abnormal, rapid, shallow breathing found in human subjects exercising during experimental O_3 exposure compared with subjects exercising in clean air (Chapter 10). Lee et al. (1979, 1980) showed that abnormal, rapid, shallow breathing in conscious dogs immediately following 2-hr exposures to 1100 to 1666 $\mu g/m^3$ (0.56 to 0.85 ppm) of O_3 was a hyperreactive airway response. This abnormal breathing pattern was elicited by mild exercise, histamine aerosol inhalation, or breathing air with reduced oxygen (O_2) or elevated carbon dioxide (CO_2) concentrations. The rapid, shallow breathing observed in dogs following O_3 exposure was not affected by drug-induced bronchodilatation (inhaled isoproteronol aerosols) or by blocking vagally induced bronchoconstriction with atropine. In all cases, rapid, shallow breathing was abolished by vagal cooling, which blocked the transmission of sensory nerves located in the airways. These investigators (Lee et al., 1979, 1980) suggest that the rapid, shallow breathing observed following O_3 exposure in dogs is caused by heightened activity of sensory nerves located in the airways. The increased reactivity of these sensory nerves is independent of smooth muscle tone (either bronchodilatation or bronchoconstriction).

Studies of lung morphology following O_3 exposure showed damage to the respiratory epithelium (Section 9.3.1). Damage to the epithelium overlying sensory receptors may be responsible for the increased receptor reactivity to mechanical stimulation (increased ventilation with exercise, low O_2, or high CO_2) or chemical (histamine) stimulation (Nadel, 1977; Boushey et al., 1980). The rapid, shallow breathing observed in guinea pigs during O_3 exposures (Amdur et al. 1978; Yokoyama, 1969; Murphy et al., 1964) may also be related to increased sensory neural activity coming from the lungs, not to an indirect effect of changes in airway diameter or lung distensibility as previously speculated.

In their study of allergic lung sensitization, Osebold et al. (1980) showed additional functional evidence for epithelial disruption caused by O_3 exposure. These investigators studied the anaphylactic response of mice to intravenous ovalbumin injection following repeated inhalations of aerosolized ovalbumin. Mice were continuously exposed to 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 for four periods of 3 to 5 days each, separated by 3 to 8 days of ambient air exposure. During periods of O_3 exposure, mice were removed from the exposure chamber for short periods, and they inhaled ovalbumin aerosols for 30 min. Mice exposed to O_3 and ovalbumin aerosols developed more severe anaphylactic reactions and had a higher incidence of fatal anaphylaxis than air-exposed mice receiving the same exposure to aerosolized ovalbumin. In mice exposed to aerosolized ovalbumin, 34 percent of the O_3-exposed mice (1568 $\mu g/m^3$; 0.8 ppm) developed fatal anaphylaxis following intravenous ovalbumin injection, compared with 16 percent of the air-exposed animals. This study also showed some indication of an interaction between O_3 exposures and exposures to sulfuric acid aerosols. Of the mice exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3 plus 1 mg/m^3 of sulfuric acid aerosols, 55 percent died of anaphylactic shock following intravenous ovalbumin injection, compared with 20-percent mortality in mice exposed to O_3 alone and zero mortality in mice exposed to sulfuric acid aerosols alone. The authors propose that these data may indicate that pollutants can increase not only the total number of clinical asthma attacks, but also the number of allergically sensitized individuals in the population. Matsumura

(1970) observed a similar increase in the allergic response of sensitized guinea pigs to inhaled antigen following 30 min of exposure to 3920 µg/m^3 (2.0 ppm) of O_3.

9.3.3 Biochemically Detected Effects

9.3.3.1 Introduction. This section will include some studies involving concentrations above 1960 µg/m^3 (1 ppm) of O_3, because the direction of the effect is opposite that for lower O_3 concentrations (decrease vs. increase in many parameters). An extensive body of data on this topic has been reviewed by Menzel (1983), Mustafa et al. (1977, 1980, 1983), Mustafa and Lee (1979), Cross et al. (1976), and Chow (1983). To facilitate presentation of this information, it has been categorized by broad classes of metabolic activity. This results in some degree of artificial separation, particularly because many patterns of response to O_3 are similar across the classes of metabolism. Lung permeability is discussed in this section, because it is typically detected biochemically. The final subsection presents hypotheses about the molecular mechanism(s) of action of O_3, relying on the data presented earlier by metabolic class.

9.3.3.2 Antioxidant Metabolism. Antioxidant metabolism of the lungs is influenced by O_3 exposure. As shown in the schematic diagram below (Figure 9-3), this system consists of a number of enzymes. As shall be discussed, O_3 has been shown to produce several reactive oxidant species *in vitro* from com-

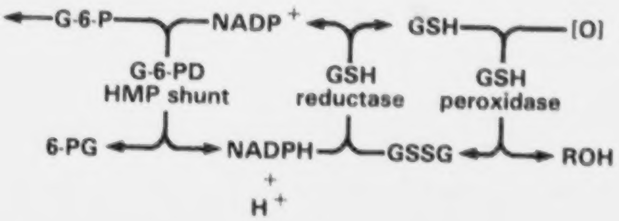

Figure 9-3. Intracellular compounds active in antioxidant metabolism of the lung. (G-6-P = glucose-6-phosphate; 6-PG = 6-phosphogluconate; G-6-PD = glucose-6-phosphate dehydrogenase; HMP shunt = hexose monophosphate shunt; NADP+ = nicotinamide adenine dinucleotide phosphate; NADPH = reduced NADP; GSH = glutathione; GSSG = glutathione disulfide; [O] = oxidizing moiety [i.e., hydrogen peroxide, free radical, lipid peroxide]; GSH peroxidase = glutathione perioxidase; GSH reductase = glutathione reductase; and ROH = reduced form of [O]).

Source: U.S. Environmental Protection Agency (1978).

pounds found in the lung, as well as in other organs. It is reasonably certain that O_3 can produce such reactive species in the lung after *in vivo* exposure. Many of these oxidant species are metabolized by the glutathione peroxidase system, rendering them less toxic. Thus, this system is involved in the toxicology of O_3.

Typically, following exposures to levels of O_3 below 1960 $\mu g/m^3$ (1 ppm), the activities of most enzymes in this system are increased (Table 9-5). Whether this increase is due to direct mechanisms (e.g., *de novo* synthesis resulting in greater enzyme activity), indirect mechanisms (e.g., an increased number of type 2 cells that naturally have a higher enzymic activity than type 1 cells), or a combination of both has not been proven. The increase in type 2 cells is most likely to be the mechanism, because with similar exposure regimens, the effects (increased enzyme activities and increased numbers of type 2 cells) increase, reaching a maximum at 3 to 4 days of exposure and a steady state on day 7 of exposure. Whatever the mechanism, the increase occurs at low O_3 levels in several species under varying exposure regimens (Table 9-5). The net result is that the antioxidant metabolism of the lung is increased. Whether this is a protective or a toxic response is often debated. To resolve this debate scientifically will require more knowledge of the mechanisms involved. However, even attributing it to be a protective response implies a physiological need for protection (e.g., an initial toxic response occurred which required protection). A more detailed discussion of these effects follows.

Acute exposures to high concentrations of O_3 generally decrease antioxidant metabolism, whereas repeated exposures to low levels increase this metabolism. For example, DeLucia et al. (1975a) compared the effects of acute (2 to 8 hr) exposures to high O_3 levels (3920 and 7840 $\mu g/m^3$, 2 and 4 ppm) and short-term (8 or 24 hr/day, 7 days) exposures to lower O_3 levels (392, 980, 1568 $\mu g/m^3$, 0.2, 0.5, 0.8 ppm) on rats. For nonprotein sulfhydryl levels (principally glutathione, GSH), decreases in the level of GSH were progressive with time of exposure (2 to 6 hr) to 7840 $\mu g/m^3$ (4 ppm). For glutathione disulfide (GSSG), decreases were less and had returned to normal by 6 hr of exposure. These exposure regimens also decreased the activities of GSH reductase and glucose-6-phosphate dehydrogenase (G-6-PD). After the first day of a 7-day continuous exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3, no significant change was seen in the nonprotein sulfhydryl or GSH content or in the activities of G-6-PD, GSH reductase, or disulfide reductase. However, the levels/ activities

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
μg/m^3	ppm					
196	0.1	NBKI	Continuous for 7 days	With vit E-deficient diet, increased levels of GSH and activities of GSH peroxidase, GSH reductase, and G-6-PD; no effect on malic dehydrogenase. With 11 ppm vit E diet, increased levels of GSH peroxidase and G-6-PD. With 110 ppm vit E diet, no change.	Rat	Chow et al., 1981
196 392	0.1 0.2	I	Continuous for 7 days	With 66 ppm vit E-supplemented diet, increase in oxygen consumption of lung homogenates only at 0.2 ppm. With 11 ppm vit E-supplemented diet, increase in O_2 consumption at 0.1 and 0.2 ppm. Increase due to increased amount of mitochondria in lungs.	Rat	Mustafa, 1975 Mustafa and Lee, 1976
196 392	0.1 0.2	MAST, NBKI	Continuous for 7 days	Increased activities of GSH peroxidase, GSH reductase, and G-6-PD and of NPSH levels with (66 mg/kg) or without (11 mg/kg) vit E supplementation; at 0.2 ppm, effects less with vit E supplementation. Morphological lesions unaffected by vit E supplementation.	Rat	Plopper et al., 1979
392 980 1568	0.2 0.5 0.8	I	Continuous for 7 days; or 8 hr/day for 7 days	For the continuous exposure to the two higher concentrations, increased activities GSH peroxidase, GSH reductase, and G-6-PD. At the lower concentration (continuous), increased activities of GSH peroxidase and GSH reductase. A linear concentration-related increase in all three enzyme activities. Increased O_2 consumption using succinate-cytochrome C reductase activity fairly proportional to O_3 level. Similar results for intermittent exposure groups.	Rat	Mustafa and Lee, 1976
1568	0.8		Continuous for 1 to 30 days	Increased rates of O_2 consumption, reaching a peak at day 4 and remaining at a plateau for the remainder of the 30 days. Also an initial decrease (day 1) and a subsequent increase (day 2) in activity of succinate-cytochrome C reductase which plateaued between days 3 to 7.		

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
μg/m³	ppm					
392 686 980 1568	0.2 0.35 0.5 0.8	I	8 hr/day for 7 days	Increased concentration-related activities of G-6-PD, NADPH-cytochrome c reductase, and succinate oxidase. Significant increases occurred in the bonnet monkey at 0.35 and 0.5 ppm; in the rhesus monkey at 0.8 ppm. However, actual data were only reported for succinate oxidase.	Monkey,	Mustafa and Lee, 1976
392 980 1568	0.2 0.5 0.8	NBKI	Continuous for 8 days or 8 hr/day for 7 days	For continuous exposure to two higher concentrations, increased activities of GSH peroxidase, GSH reductase, and G-6-PD. At the lower concentration (continuous), increased activities of GSH peroxidase and GSH reductase. A concentration-related linear increase in all three enzyme activities. Similar results obtained for intermittent exposure groups.	Rat	Chow et al., 1974
392 980 1568	0.2 0.5 0.8	MAST, NBKI	8 or 24 hr/day for 7 consecutive days	Activities of G-6-PD and NADPH-cytochrome C reductase and succinate oxidase increase in a concentration-dependent fashion. No significant differences between the intermittent and continuous exposure groups.	Rat	Schwartz et al., 1976
392 980 1960	0.2 0.5 1.0	NBKI	3 hr/day for 4 days	Reduced glutathione levels increased in a linear concentration-dependent manner. No effect at 0.2 ppm in the no-exercise group. Exercise enhanced effect.	Mouse	Fukase et al., 1978
392 980 1960	0.2 0.5 1.0	ND	4 hr/day for up to 30 days	GSH content increased directly with O_3 concentration and exposure duration. Increase in activities of G-6-PD, GSH reductase, and GSH peroxidase after 7 days of exposure to 0.5 and 1 ppm.	Mouse	Fukase et al., 1975
2352- 16,072	1.2- 8.2		4 hr	Decrease in GSH content after exposure to 8.2 ppm. No change below 4.0 ppm. Two days postexposure to 4 ppm, GSH content increased, lasting for several days.		

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE (continued)

Ozone concentration $\mu g/m^3$	ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
392 980 1568	0.2 0.5 0.8	MAST, NBKI	8 or 24 hr/day for 7 days	All O_3 levels: increase in NPSH levels; increased activities of G-6-PD, GSH reductase, NADH cyt. c reductase. At 0.5 and 0.8 ppm, increased activity of succinate cyt. c reductase. At 0.8 ppm, continuous increase began at day 2 of exposure.	Rat	DeLucia et al., 1975a
1568	0.8		8 hr/day for 7 days	Increased NPSH, GSH, and G-6-PD; no change in other enzymes.	Monkey	
3920 7840	2 4		2 to 8 hr	Loss of GSH; loss of SH from lung mitochrondrial and microsomal fractions and inhibition of marker enzyme activities from these fractions.	Rat	
392 980 1568	0.2 0.5 0.8	I	Continuous for 7 days	Concentration-related increase in O_2 consumption.	Rat	Mustafa et al., 1973
627	0.32	UV	6 hr	In both vit E-supplemented and nonsupplemented groups: increased G-6-PD activities and GSH levels; decreased ACHase activities.	Mouse	Moore et al., 1980
882	0.45	UV	Continuous for 5 days	Mice: increased levels/activities of TSH, NPSH, GSH peroxidase, GSH reductase, G-6-PD, 6-P-GD, isocitrate dehydrogenase, cytochrome c oxidase, and succinate oxidase. Rats: increased levels/activities of NPSH, GSH peroxidase, and G-6-PD in several strains. Generally mice were more responsive. For both species, no change in DNA or protein levels or activity of GSH-S-transferase.	Mouse, 3 strains of rats	Mustafa et al., 1982
882	0.45	UV	8 hr/day for 7 days	O_3 and 4.8 ppm of NO_2 alone produced no significant effects but O_3 + NO_2 produced synergistic effects: increased total and nonprotein sulfhydryls; increased activities of succinate oxidase and cytochrome c	Mouse	Mustafa et al., 1984

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
µg/m³	ppm					
882	0.45	ND	Continuous for 7 days	Increased SOD activity at days 3 and 5, but not days 2 and 7 of exposure.	Rat	Bhatnagar et al., 1983
980	0.5	NBKI	8 hr/day for 7 days	Increases in activities of GSH peroxidase, GSH reductase and G-6-PD and in GSH levels of rats. No effect in monkeys.	Rat Monkey	Chow et al., 1975
1372	0.7	NBKI	Continuous for 5 days	Increased activities of GSH peroxidase, G-6-PD, and GSH reductase. Malonaldehyde observed.	Rat	Chow and Tappel, 1972
1568	0.8		Continuous for 7 days	The increases in the first two enzymes were partially inhibited as a logarithmic function of vitamin E levels in diet.		
1470	0.75	NBKI	Continuous for up to 30 days	Increase in activities of GSH peroxidase, GSH reductase, G-6-PD, 6-P-GD, and pyruvate kinase at day 3, reaching a peak at day 10, at which time beginning of a slight decrease (except for GSH peroxidase which continued to increase). At day 30 still elevated over controls.	Rat	Chow and Tappel, 1973
1568	0.8		Continuous for 7 days	Increased activities of hexose monophosphate shunt and glycolytic enzymes of lung.		
1568	0.8	NBKI	Continuous for 3 days	Increased activities of GSH peroxidase, GSH reductase, and G-6-PD; levels of NPSH; general protein synthesis; and rate of mitochondrial succinate oxidation. Decrease to control values 6 to 9 days postexposure. Re-exposure using same regimen (6, 13, or 27 days postexposure) resulted in similar elevations.	Rat	Chow et al., 1976b
1568	0.8	I	Continuous for 10 to 20 days	At the higher concentration: increase in the lung mitochondrial O_3 consumption in oxidation of 2-oxyglutarate and glycerol-1-phosphate and the number of type 2 alveolar cells which are rich in mitochondria. No change in malonaldehyde. At the lower concentration: increase in O_2 consumption.	Rat	Mustafa et al., 1973
3920	2		8 hr			

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE (continued)

Ozone concentration $\mu g/m^3$	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
1568	0.8	I	< 24 hr	NPSH level unaffected at 0.8 ppm of O_3 for < 24 hr or or 1.5 ppm for < 8 hr; decreased at 2 ppm of O_3 for 8 hr or 4 ppm for 6 hr. At 4 ppm of O_3 for 6 hr, decreased level of GSH; no change in GSSG level.	Rat	DeLucia et al., 1975b
2940-7840	1.5-4		< 8 hr			
1568	0.8	I	10 days	Lung SH levels unchanged. Increase in G-6-PD and and cytochrome c reductase activities. No change in malonaldehyde levels.	Rat	DeLucia et al., 1972
3920	2		4 to 8 hr	Decrease in lung SH levels and in G-6-PD, GSH reductase, and cytochrome c reductase activities. No change in malonaldehyde levels.		
3920-5880	2-3		30 min	In vitro: decrease in SH levels; increase in malonaldehyde levels.		
1568	0.8	ND	Continuous for 7 days	Increased activity of superoxide dismutase.	Rat	Mustafa et al., 1977
1568	0.8	UV	72 hr	Succinate oxidase, cytochrome c oxidase, and isocitrate dehydrogenase: No effect at 24 days old, increased in 90-day-old rats. G-6-PD, 6-PGH: increased at 24 and 90 days of age, latter had greater increase. Succinate oxidase and G-6-PD decreased in 7- and 12-day-old rats and increased in 18-day-old rats. High mortality in 7- and 12-day-old rats.	Rat (7 to 90 days old)	Elsayed et al., 1982a
1568	0.8	UV	Continuous for 5 days	Diet was constant vitamin E and deficient or supplemented (2 levels) with selenium (Se). No change in GSH peroxidase. With 0 Se, decreased GSH reductase activity; no change with low or high Se. Progressive increase in activities of G-6-PD and 6-P-GD with increasing Se, beginning at low Se level.	Mouse	Elsayed et al., 1982b

TABLE 9-5. CHANGES IN THE LUNG ANTIOXIDANT METABOLISM AND OXYGEN CONSUMPTION BY OZONE (continued)

Ozone concentration μg/m³	ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
1568	0.8	UV	Continuous for 5 days	Diet was constant vitamin E and 0 ppm of Se or 1 ppm in Se. +Se: increased G-6-PD, 6-P-GD; no change in GSH reductase or GSH peroxidase. -Se: decreased GSH reductase. Both diet groups had increase in TSH and NPSH, and lung Se levels after O_3.	Mouse	Elsayed et al., 1983
1568	0.8	NBKI	continuous for 7 days	Vitamin E partially prevented increased activities of G-6-PD, 6-P-GD, and malic enzyme. Activities of phosphofructokinase and pyruvate kinase increased. No effect on aldolase and malate dehydrogenase.	Rat	Chow and Tappel, 1973
1764	0.9	CHEM	96 hr	Trend towards decreased activities of GSH reductase GSH peroxidase, G-6-PD before 18 days of age, followed by increases thereafter. For G-6-PD: no change at 5 and 10 days of age; decrease at 15 days, and increase at 25 and 35 days.	Rat (5-180 days old)	Tyson et al., 1982
1764	0.9	MAST	$\geq$ 96 hr	96 hr: No effect below 20 days of age; G-6-PD increases thereafter up to 35 days, after which (40 and 50 days old) it decreases. When exposure started at 25 or 32 (but not 10 to 15) days of age, the maximal increase in G-6-PD occurred at about 32 days of age under continuous exposure conditions.	Rat (10-50 days old)	Lunan et al., 1977

[a] Measurement method: MAST = KI-coulometric (Mast meter); NBKI = neutral buffered potassium iodide; CHEM = gas solid chemiluminescence; UV = UV photometry; I = iodometric; ND = not described.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] Abbreviations used: GSH = glutathione; GSSG = reduced glutathione; G-6-PD = glucose-6-phosphate dehydrogenase; LDH = lactate dehydrogenase; NPSH = non-protein sulfhydryls; SH = sulfhydryls; 6-P-GD = 6-phosphogluconate dehydrogenase.

of these constituents increased by day 2 and remained elevated for the remainder of the exposure period. Comparable results were reported with similar (but not identical) exposure regimens by DeLucia et al. (1972, 1975b) using rats and Fukase et al. (1975) using mice.

Investigators (Table 9-5) have found that for lower levels of O_3, increases in antioxidant metabolism are linearly related to O_3 concentration. Most such studies were conducted by using intermittent and continuous exposures. No differences between these regimens were found, suggesting that concentration of exposure is more important than time of exposure.

Chow et al. (1974) exposed rats continuously or intermittently (8 hr/day) for 7 days to 392, 980, or 1568 $\mu g/m^3$ (0.2, 0.5, or 0.8 ppm) of O_3 and found a concentration-related linear increase in activities of GSH peroxidase, GSH reductase, and G-6-PD. Significant increases occurred for all measurements, except G-6-PD at continuous exposure to 392 $\mu g/m^3$ (0.2 ppm). Although the difference between continuous and intermittent exposure was not examined statistically, no major differences appeared to exist. Schwartz et al. (1976) made similar observations for G-6-PD activity when using identical exposure regimens and found concurrent morphological changes (Section 9.3.1). Mustafa and Lee (1976), also by using identical exposure regimens, found similar effects for G-6-PD activity. DeLucia et al. (1975a) found similar changes and increased nonprotein sulfhydryls at all three concentrations of O_3. A similar study was performed in mice by using a longer exposure period of 30 days (Fukase et al., 1975). The increase in GSH level was related to concentration and time of exposure. Fukase et al. (1978) also observed a linear concentration-related increase in GSH levels of mouse lungs exposed 3 hr/day for 4 days to 392, 980, or 1960 $\mu g/m^3$ (0.2, 0.5, or 1.0 ppm) of O_3. Exercise enhanced the effect. At the lower O_3 level, the increase in GSH was significant only in the exercising mice.

The influence of time of exposure was examined directly by Chow and Tappel (1973). Rats were exposed continuously to 1470 $\mu g/m^3$ (0.75 ppm) for 1, 3, 10, or 30 days, at which times measurements of GSH reductase, GSH peroxidase, G-6-PD, pyruvate kinase, and 6-phosphogluconate dehydrogenase activities were made. No statistical tests or indications of data variability were presented. A few of the enzyme activities (GSH peroxidase and 6-phosphogluconate dehydrogenase) may have decreased at day 1 of exposure. All enzyme activities except GSH peroxidase increased by day 3 and reached a peak at 10 days and then began to return toward control values. GSH peroxidase activity continued to increase over this time of exposure. In a similar study (Mustafa and Lee, 1976), G-6-PD activity was measured after rats were exposed for 7 days to 1568 μg/m^3 (0.8 ppm) continuously. No effect was detected on day 1, but by day 2 the activity had increased. The peak response was on day 4; the activity remained elevated to an equivalent degree on day 7. In a similar experiment, DeLucia et al. (1975a) obtained equivalent results.

The tolerance phenomenon has also been investigated for lung antioxidant metabolism. Rats were exposed continuously for 3 days to 1568 μg/m^3 (0.8 ppm) of O_3, allowed to remain unexposed for 6, 13, or 27 days, and then re-exposed for 3 days to the same O_3 level (Chow et al., 1976b). Immediately after the first 3 days of exposure, the activities of GSH peroxidase, GSH reductase, and G-6-PD were increased, as was the nonprotein sulfhydryl content. By 2 days after this exposure ceased, recovery had begun; control values were completely reached by 9 days postexposure. Following a 30-day recovery period, no changes were observed. When re-exposure commenced on day 6 of recovery (at which time incomplete recovery was observed), the metabolic activities returned to levels equivalent to those of the original exposures. Similar findings were made when re-exposure commenced on days 13 and 27 days of the recovery period.

The influence of vitamin E, an antioxidant, on O_3 toxicity has been extensively studied, because it typically reduces the toxicity of O_3 in animals. This topic has been recently reviewed by Chow (1983). Early studies centered on mortality. For example, vitamin E-deficient rats are more susceptible to continuous exposure to 1960 μg/m^3 (1 ppm) of O_3 than rats fed supplements of vitamin E (LT50, the time at which a 50 percent mortality is observed, 8.2 days versus 18.5 days) (Roehm et al., 1971a, 1972). Vitamin E protected animals from mortality and changes in the wet to dry weight ratios of the lung (lung edema) on continuous exposure to 1568 μg/m^3 (0.8 ppm) of O_3 or higher for 7 days (Fletcher and Tappel, 1973). Vitamin E protection against O_3 is positively correlated to the log concentration of dietary vitamin E fed to the rats. Rats maintained on vitamin E-supplemented diets and exposed to 1568 μg/m^3 (0.8 ppm) of O_3 continuously for 7 days also had changes in 6-phosphogluconate dehydrogenase activity. Rats were exposed to 1372 to 31,360 μg/m^3 (0.7 to 16 ppm) of O_3 while being fed diets containing ascorbic acid, dl-methionine, and butylated hydroxytoluene (Fletcher and Tappel, 1973). This combination was supposed to be a more potent antioxidant mixture than vitamin E alone. Animals fed diets with the highest level of this antioxidant mixture had the greatest survival rate. Animals fed α-tocopherol (vitamin E) in the range of 10 to 150 mg/kg of diet had a survival rate slightly lower than those fed the combination of antioxidants.

Donovan et al. (1977) fed mice 0 (deficient diet), 10.5 (minimal diet), or 105 (supplemental diet) mg/kg of vitamin E acetate. The diet was also altered to increase the peroxidizability of the lung by feeding either low or high polyunsaturated fats (PUFA). Mice were continuously exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3. The mortality (LT50 of 29 to 32 days) was the same, regardless of the large differences in peroxidizability of the lungs of animals fed high- or low-PUFA diets. High supplemental levels (105 mg/kg) of vitamin E acetate were protective and delayed the LT50 to O_3 by an average of 15 days. Although these experiments demonstrate clearly the protective effect of vitamin E against O_3 toxicity, they do not support the hypothesis that changes in fatty acid composition of the lung will increase O_3 toxicity. The results could be interpreted to indicate that the scavenging of radicals by vitamin E is more important than the relative rate of oxidation of PUFA. These findings led to biochemical studies that used graded levels of dietary vitamin E.

Plopper et al. (1979) correlated biochemical and morphological (Section 9.3.1) effects in rats maintained on a synthetic diet with 11 mg kg/vitamin E (equivalent to the average U.S. adult intake) or commercial rat chow having 66 mg/kg vitamin E. The 11 mg/kg vitamin E group was exposed continuously for 7 days to 196 or 392 $\mu g/m^3$ (0.1 or 0.2 ppm) of O_3, and measurements were made at the end of exposure. The rats on the commercial diet were exposed to only the higher concentration. All exposures increased activities of GSH peroxidase, GSH reductase, and G-6-PD, and the amount of nonprotein sulfhydryl. Although statistical comparisons between the dietary groups were not made, greater increases appear to have occurred in the 11 mg/kg vitamin E group; the magnitude of the responses in the higher vitamin E group at 392 $\mu g/m^3$ (0.2 ppm) of O_3 was roughly equivalent to the magnitude of the responses of the low vitamin E group exposed to 196 $\mu g/m^3$ (0.1 ppm). The 2 dietary groups showed little variation in morphological effects.

These studies were expanded to include three vitamin E dietary groups: 0, 11, or 110 ppm (Chow et al., 1981). Rats were exposed to 196 $\mu g/m^3$ (0.1 ppm) of O_3 continuously for 7 days. In the 0-ppm vitamin E group, O_3 increased the level of GSH and the activities of GSH peroxidase, GSH reductase, and G-6-PD. Increases of similar magnitude occurred in the 11-ppm vitamin E group, with the exception of GSH reductase activity, which was not affected.

In the highest vitamin E group, no significant effects were observed. Ozone caused no changes in the activity of malic dehydrogenase in any of the dietary groups. Morphologically (Section 9.3.1), only 1 of 6 rats of the 110-ppm vitamin E group had lesions, whereas more rats of the two other groups had lesions. These lesions became more severe as the vitamin E level decreased. They occurred at the bronchio-alveolar junction and were characterized by disarrangement of the bronchiolar epithelium and an increase in the number of alveolar macrophages.

Chow and Tappel (1972) exposed rats continuously to 1372 $\mu g/m^3$ (0.7 ppm) of O_3 for 5 days. The animals had been maintained on diets with different levels of dl-α-tocopherol acetate (vitamin E) (0, 10.5, 45, 150, and 1500 mg/kg diet). Ozone exposure increased GSH peroxidase, GSH reductase, and G-6-PD activities. For GSH peroxidase and G-6-PD activities, the increase was reduced as a function of the logarithmic concentration of vitamin E. Vitamin E did not alter the magnitude of the effect on GSH reductase, a finding in contrast to the results of others (Chow et al., 1981; Plopper et al., 1979). Malonaldehyde, which is produced by lipid peroxidation, increased; this increase was also partially inhibited as a logarithmic function of vitamin E concentration. However, others (DeLucia et al., 1972; Mustafa et al., 1973) have not observed the presence of malonaldehyde in exposed lungs. The increase in malonaldehyde and activity of GSH peroxidase were linearly correlated, leading Chow and Tappel (1972) to propose a compensatory mechanism in which the increase in GSH peroxidase activity increases lipid peroxide catabolism.

Chow and Tappel (1973) observed the typical protection of vitamin E (0 and 45 mg/kg diet α-tocopherol) from the effect of O_3 (1568 $\mu g/m^3$, 0.8 ppm; 7 days, continuous) on increasing G-6-PD activity in rat lungs. Similar findings occurred for 6-phosphogluconate dehydrogenase and malic enzyme activities. The activities of two glycolytic regulating enzymes, phosphofructokinase and pyruvate kinase, were increased by O_3 exposure but were not influenced by vitamin E levels in the diet. Aldolase and malate dehydrogenase activities were not affected.

Elsayed et al. (1982b, 1983) examined the influence of selenium (Se) in the diet on GSH peroxidase activity in the lung. Selenium is an integral part of one form of the enzyme GSH peroxidase. Mice were raised on a diet containing 55 ppm vitamin E with either 0 ppm or 1 ppm of Se and exposed to 1568 ± 98 $\mu g/m^3$ (0.8 ppm) of O_3 continuously for 5 days. In these mice, Se deficiency caused a sevenfold decline in Se level and a threefold decline in GSH peroxidase activity in the lung. Other enzyme activities (e.g., GSH reductase, G-6-PD, 6-phosphogluconate dehydrogenase) were not affected by dietary Se. After O_3 exposure, the GSH peroxidase activity in the Se-deficient group remained unstimulated and was associated with a lack of stimulation of GSH reductase, G-6-PD, and 6-phosphogluconate dehydrogenase activities. In contrast, the O_3-exposed Se-supplemented group exhibited increases in 6-phosphogluconate dehydrogenase and G-6-PD activities. Dietary deficiency or supplementation of Se, vis-a-vis alteration of GSH peroxidase activity, did not appear to influence the effects of O_3 exposure as assessed by other parameters. Although the animals received the same level of dietary vitamin E, after air or O_3 exposure, the Se-deficient group showed a two-fold increase in lung vitamin E levels relative to the Se-supplemented group, suggesting a complementary relationship between Se and vitamin E in the lung. This sparing action between Se (i.e., GSH peroxidase activity) and vitamin E might explain similar effects of O_3 exposure in Se-deficient and supplemented mice.

Several investigators have studied the responsiveness of different species to the effect of O_3 on antioxidant metabolism. DeLucia et al. (1975a) exposed both Rhesus monkeys and rats for 7 days (8 hr/day) to 1568 $\mu g/m^3$ (0.8 ppm). The nonprotein sulfhydryl and GSH content were increased, as was G-6-PD activity. Activity of GSH reductase was affected in the rats but not the monkeys. No statistical comparisons were made between the rats and monkeys. In the only parameter for which sufficient data were presented for comparison, G-6-PD, the increase in monkeys was about 125 percent of controls; for rats, it was about 130 percent of controls.

Rats and Rhesus monkeys were compared more extensively by Chow et al. (1975). Animals were exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3 8 hr/day for 7 days. The nonprotein sulfhydryl content and the activities of GSH peroxidase, GSH reductase, and G-6-PD increased in rats but not in monkeys. The magnitude of the increases in rats was 20 to 26 percent. The increases in monkeys were between 10 and 15 percent and statistically insignificant, "because of relatively large variations," according to the authors. The variation in the monkeys was approximately double that of the rats. The sample size of the monkeys (6) was lower than that of the rats (8). Statistical tests of the Type II error (e.g., false negative error) rates were not reported. Thus, the monkeys apparently were not affected to the same degree as the rats. However, the experiments with monkeys were apparently not conducted with as much statistical power as those with rats. Thus, under the actual study designs used, ozone would have had to have substantially greater effects on monkeys than rats for a statistically significant effect to be detected. This did not happen, leading to the conclusion of the investigators that monkeys are not more responsive. Studies of improved experimental design would indicate more definitively whether monkeys are less responsive. Mustafa and Lee (1976) also alluded to different G-6-PD responses of rats and Bonnet and Rhesus monkeys after exposures for 8 hr/day for 7 days to levels as low as 392 $\mu g/m^3$ (0.2 ppm). However, no data for G-6-PD were presented, and the description of these results was incomplete.

Mice (Swiss Webster) and 3 strains of rats (Sprague-Dawley, Wistar, and Long Evans) were compared after a 5-day continuous exposure to 882 $\mu g/m^3$ (0.45 ppm) of O_3 (Mustafa et al., 1982). Total sulfhydryl content increased only in mice. However, nonprotein sulfhydryl content increased in both rats and mice to a roughly equivalent degree. GSH-S-transferase was not affected in any of the animals. Mice exhibited the typical increases in the activities of GSH peroxidase, GSH reductase, G-6-PD, 6-phosphogluconate dehydrogenase, and isocitrate dehydrogenase. Rats were less affected; no changes were seen in the activities of GSH reductase or isocitrate dehydrogenase, and not all strains of rats showed an increase in the activities of GSH peroxidase, G-6-PD, and 6-phosphogluconate dehydrogenase. For GSH reductase and G-6-PD, the increased activities in exposed mice were significantly greater than those in exposed rats.

At present, it is not possible to determine whether these apparent species differences in responsiveness were due to differences in the total deposited dose of O_3, an innate difference in species sensitivity, or differences in experimental design (e.g., small sample sizes, insufficient concentration-response studies).

Age-dependent responsiveness to O_3-induced changes in GSH systems has been observed. Tyson et al. (1982) exposed rats (5 to 180 days old) to 1764 $\mu g/m^3$ (0.9 ppm) of O_3 continuously for 96 hr, except for suckling neonates (5 to 20 days old) which received an intermittent exposure (4 hr of exposure, 1.5 hr no exposure, 4 hr exposure). Given that others (Mustafa and Lee, 1976; Chow et al., 1974; Schwartz et al., 1976) have observed no differences between continuous and intermittent exposures for these enzymatic activities, this difference in regimen can be considered inconsequential. All ages given are ages at the time of initiation of exposure. They were calculated from those given in the report to facilitate comparisons with other reports on age sensitivity. Weanlings (25 and 35 days old) and nursing dams (57 and 87 days old) had higher lung to body weight ratios. Generally, the DNA content of the younger animals was unchanged. When the activities of G-6-PD, GSH reductase, and GSH peroxidase were measured after O_3 exposure, the trend was a decrease in activities at and before 18 days of age, followed by increases thereafter. For G-6-PD, this trend was most pronounced; at 5 and 10 days of age, no significant changes were seen; at 15 days of age, a decrease was seen; and at 25 and 35 days of age, progressively greater increases were seen.

Ten- to 50-day-old rats were exposed continuously to 1764 $\mu g/m^3$ (0.9 ppm) of O_3 (Lunan et al., 1977). Ages of rats reported are presumably ages at initiation of exposure. In rats (10 to 40 days old) exposed for 3 days, G-6-PD activity was measured periodically during exposure. No statistical analyses were reported. Ozone caused a possible increase (20 percent of control) in the activity of G-6-PD in the 20-day-old group, and the magnitude continued to increase as age increased up to about 35 days (~ 75 percent of control), after which (40 and 50 days of age) the effect became less (40 percent of control at 50 days of age). In another experiment, a complex design was used in which rats at 10, 15, 25, and 32 days of age were exposed up to 32 to 34 days of age; thus, the duration of exposure for each group was different. When the animals were younger than 20 days, no effect was observed. When older mice were used, the greatest magnitude of the increased activity occurred at about 32 days of age, regardless of the absolute length of exposure.

Elsayed et al. (1982a) exposed rats of various ages to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 continuously for 72 hr. Ages given are those at initiation of exposure. Ozone increased lung weights, total lung protein, and total lung DNA in an age-dependent fashion, with the older (90-day-old) rats being more affected than 24-day-old animals. For isocitrate dehydrogenase activity, no effect was seen in the 24-day-old rats, but an increase was observed in the 90-day-old animals. For G-6-PD and 6-phosphogluconate dehydrogenase, increases were observed in the 24- and 90-day-old rats, with a greater magnitude of the effect occurring in the 90-day-old group. Younger rats (7 to 18 days old) were also examined. The exposure caused >60 percent mortality to the 7- and 12-day-old rats. Glucose-6-phosphate dehydrogenase activity decreased in both the 7-and-12-day old groups, with the younger rats being more affected. The 18-day-old rats had an increase in this activity. These trends were similar to those observed by Tyson et al. (1982), although the exact age for significant changes differed slightly.

The reason for these age-dependent changes is not known. The younger animals (< 24 days of age) have lower basal levels of the studied enzymes than the older animals examined (> 38 days of age) (Tyson et al., 1982; Elsayed et al., 1982a). It is conceivable that age influenced the dosimetry of O_3. The decreased activities observed in the neonates are reminiscent of the decreased activities that occur at higher O_3 levels in adults (DeLucia et al., 1972, 1975a; Fukase et al., 1975). The increased activities in the later stages of weanlings or in young, growing adults is consistent with the effects observed in other studies of adult rats (Table 9-5).

Mustafa et al. (1984) are the only researchers to report the effects of combined exposures to O_3 and nitrogen dioxide on the GSH peroxidase system. Mice were exposed 8 hr/day for 7 days to either 9024 $\mu g/m^3$ (4.8 ppm) of nitrogen dioxide, or 882 $\mu g/m^3$ (0.45 ppm) of O_3, or a mixture of these. Ozone and nitrogen dioxide alone caused no significant effects on most of the endpoints; however, synergistic effects were observed in the mixture group. The total sulfhydryl and nonprotein sulfhydryl contents were increased. The activities of GSH peroxidase, G-6-PD, 6-phosphogluconate dehydrogenase, and isocitrate dehydrogenase increased, but the activities of GSH reductase and GSH S-transferase were unchanged. Tissue O_2 utilization was also increased, as shown by the increase in succinate oxidate and cytochrome c oxidase activities.

Superoxide dismutase (SOD) catalyzes the dismutation of (and therefore destroys) superoxide (O_2-), a toxic oxidant species thought to be formed from O_3 exposure, and is thus involved in antioxidant metabolism. Rats exposed continuously for 7 days to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 exhibited an increased activity of SOD in cytosolic and mitochondrial fractions of the lungs (Mustafa et al., 1977). In a more complex exposure regimen in which rats were exposed for 3 days to 1568 $\mu g/m^3$ (0.8 ppm) and then various days of combinations of 2940 $\mu g/m^3$ (1.5 ppm) and 5880 $\mu g/m^3$ (3 ppm) of O_3, SOD activity also increased. Bhatnager et al. (1983) studied the time course of the increase in SOD activity after continuous exposure of rats to 882 $\mu g/m^3$ (0.45 ppm) of O_3. On day 2 of exposure, there was no effect. On days 3 and 5, activity had increased; by day 7 of exposure, values were not different from control.

9.3.3.3. Oxidative and Energy Metabolism. Mitochondrial enzyme activities are typically studied to evaluate effects on O_2 consumption, which is a fundamental parameter of cellular metabolism. Mitochondria are cellular organelles that are the major sites of O_2 utilization and energy production. Many of the enzymes in mitochondria have functional sulfhydryl groups, which are known to be affected by O_3, and mitochondrial membranes have unsaturated fatty acids that are also susceptible to O_3. The patterns of O_3 effects on O_2 consumption, as will be discussed, are quite similar to effects on antioxidant metabolism (Section 9.3.3.2).

Mustafa et al. (1973) showed in rats that acute (8-hr) exposure to a high concentration of O_3 (3920 $\mu g/m^3$, 2 ppm) decreases O_2 consumption using the substrates succinate, α-oxoglutarate, and glycerol-1-phosphate. Similar findings were made by DeLucia et al. (1975a). Decreases in mitochondrial total sulfhydryl levels were also observed (Mustafa et al., 1973). Equivalent changes occurred in whole-lung homogenate and the mitochondrial fraction. No change in malonaldehyde levels was found. When rats were exposed to high O_3 levels (5880 $\mu g/m^3$, 3 ppm; 4 hr), the immediate depression in succinate oxidase activity was followed by an increase that peaked about 2 days postexposure and returned to normal by 20 days postexposure (Mustafa et al., 1977). A 10- or 20-day continuous exposure to a lower O_3 concentration (1568 $\mu g/m^3$, 0.8 ppm) caused an increase in O_2 consumption of lung homogenate which was greater at 20 days (Mustafa et al., 1973). When the activity of the mitochondrial fraction per mg of protein was measured, the increased activity was less than that of the lung homogenate per mg of protein. Morphological comparisons indicated that the exposed lungs had a threefold increase in type 2 cells, which contain more mitochondria than type 1 cells. Thus, the increase in O_2 consumption appears to reflect changes in cell populations.

Schwartz et al. (1976) exposed rats for 7 days continuously or intermittently (8 hr/day) to 392, 980, or 1568 $\mu g/m^3$ (0.2, 0.5, or 0.8 ppm) of O_3. Succinate oxidase activity increased linearly with O_3 concentration. No major differences were apparent between continuous and intermittent exposures. No statistical analyses were reported. Concentration-dependent morphological effects were also observed (Section 9.3.1). When using rats and an identical exposure regimen, Mustafa and Lee (1976) found similar responses for succinate oxidase and succinate-cytochrome c reductase activity. These increases were statistically significant. Mustafa et al. (1973), when using 7 days of continuous exposure, also showed that O_2 consumption of rats increased with increasing O_3 level (392, 980, 1568 $\mu g/m^3$, 0.2, 0.5, 0.8 ppm).

Although concentration appears to be a stronger determinant of the effect, time of exposure also plays a role (Mustafa and Lee, 1976). Rats were exposed to 1568 μg/m^3 (0.8 ppm) of O_3 continuously for 30 days, and O_2 consumption was measured as the activities of succinate oxidase, 2-oxoglutarate oxidase, and glycerol-1-phosphate oxidase. On day 1, the effect was not significant. However, at day 2 and following, these enzyme activities increased. The peak increase occurred on day 4 and remained at that elevated level throughout the 30 days of exposure. Mitochondrial succinate-cytochrome c reductase exhibited a similar pattern under similar O_3 levels for 7 days of exposure. Equivalent results occurred in rats during a 7-day continuous exposure to 1568 μg/m^3 (0.8 ppm) of O_3 (DeLucia et al., 1975a).

In rats, recovery from an ozone-induced (1568 μg/m^3, 0.8 ppm; 3 days, continuous) increase in succinate oxidase activity occurred by 6 days post-exposure (Chow et al., 1976b). When the rats were re-exposed to the same exposure regimen at 6, 13, and 27 days of recovery, the increased activity was equivalent to that of the initial exposure. Thus, no long-lasting tolerance was observed.

Dietary vitamin E can also reduce the effects of O_3 on O_2 consumption. After 7 days of continuous exposure to 196 or 392 μg/m^3 (0.1 or 0.2 ppm) of O_3, the lung homogenates of rats maintained on diets with either 11 or 66 ppm of vitamin E were examined for changes in O_2 consumption (succinate oxidase activity) (Mustafa, 1975; Mustafa and Lee, 1976). In the 11-ppm vitamin E group, increases in O_2 consumption occurred at both O_3 levels. In the 66-ppm vitamin E group, only 392 μg/m^3 (0.2 ppm) of O_3 caused an increase. Mitochondria were isolated from the lungs and studied. Neither dietary group had O_3-induced changes in the respiratory rate of mitochondria (on a per mg of protein basis). However, the amount of mitochondria (measured as total protein content of the mitochondrial fraction of the lung) from the O_3-exposed rats of the 11-ppm vitamin E group did increase (15-20 percent).

Similar to earlier discussions for antioxidant metabolism (Section 9.3.3.2), responsiveness to effects of O_3 on O_2 consumption is age-related (Table 9-5). Elsayed et al. (1982a) exposed rats of various ages to 1568 μg/m^3 (0.8 ppm) of O_3 continuously for 72 hr. The O_3-induced increase in the activities of succinate oxidase and cytochrome c oxidase increased with age (from 24 to 90 days of age), with no significant change at 24 days of age. When younger rats were examined, succinate oxidase activity decreased in both the 7- and 12-day-old animals, with the younger ones more affected. The 18-day-old rats had an increase in this activity.

Species and strain differences were observed after a 5-day continuous exposure to 882 $\mu g/m^3$ (0.45 ppm) of O_3 (Mustafa et al., 1982). Mice and Sprague Dawley rats (but not other strains of rats) had an increase in activity of succinate oxidase. Cytochrome c oxidase was increased in mice, Long-Evans rats, and Sprague Dawley rats, but not Wistar rats; the increase in the mice was greater than that in the rats.

Rats have also been compared to two strains of monkeys after a 7-day (8 hr/day) exposure to various concentrations of O_3 (Mustafa and Lee, 1976). Rats were exposed to 392, 980, or 1568 $\mu g/m^3$ (0.2, 0.5, or 0.8 ppm) of O_3 and exhibited increases in succinate oxidase activity. Rhesus monkeys exposed to either 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 had an increase in this enzyme activity only at the higher exposure concentration. When Bonnet monkeys were exposed to 392, 686, or 980 $\mu g/m^3$ (0.2, 0.35, or 0.5 ppm) of O_3, succinate oxidase activity increased at the two higher O_3 levels. The number of animals used was not specified, which makes interpretation difficult. At the 392-$\mu g/m^3$ (0.2 ppm) level, the increase in rats was to 118 percent of controls (significant); in Bonnet monkeys, it was to 113 percent of controls (not significant). At the 980-$\mu g/m^3$ (0.5 ppm) of O_3 level, the magnitude of the significant increases was not different between rats (133 percent of controls) and Bonnet monkeys (130 percent of controls). Rhesus monkeys may have been slightly less responsive than rats, but no statistical analyses were performed to assess this question.

The increase in levels of nonprotein sulfhydryls, antioxidant enzymes, and enzymes involved in O_2 consumption is typically attributed to concurrent morphological changes (Section 9.3.1) in the lungs, principally the loss of type 1 cells and the increase of type 2 cells and the infiltration of alveolar macrophages. Several investigators have made such correlated observations in rats (Plopper et al., 1979; Chow, et al., 1981; Schwartz et al., 1976; DeLucia et al., 1975a). Type 2 cells are more metabolically active than type 1 cells and have more abundant mitochondria and endoplasmic reticula. This hypothesis is supported by the findings of Mustafa et al. (1973), Mustafa (1975), and DeLucia et al. (1975a). For example, succinate oxidase was studied in both lung homogenates and isolated mitochondria of rats after a 7-day exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 (DeLucia et al., 1975a). The increase in the homogenate was about double that of the isolated mitochondria (on a per mg of protein basis). As mentioned previously, this indicates that an increase in the number of mitochondria, rather than an increased activity within a given mitochondrion, in exposed lungs is the probable dominant cause.

9.3.3.4 Monooxygenases. Multiple microsomal enzymes function in the metabolism of both endogenous (e.g., biogenic amines, hormones) and exogenous (xenobiotic) substances. These substrates are either activated or detoxified, depending on the substrate and the enzyme. Only a few of the enzymes have been studied subsequent to O_3 exposure (Table 9-6).

Monoamine oxidase (MAO) activity has been investigated (Mustafa et al., 1977) in view of its importance in catalyzing the metabolic degradation of bioactive amines like 5-hydroxytryptamine and norepinephrine. Although MAO activity is located principally in the mitochondria, it also is found in microsomes. Activity levels of MAO in rats were determined after exposure to 3920 $\mu g/m^3$ (2 ppm) for 8 hr or 1568 $\mu g/m^3$ (0.8 ppm) continuously for 7 days. Substrates used included n-amylamine, benzylamine, tyramine, and 3-hydroxytyramine; three tissue preparations were used (whole lung homogenate, mitochondria, and microsomes). The acute high-level exposure reduced MAO activity in all tissue preparations. The longer exposure to 1568 $\mu g/m^3$ (0.8 ppm) increased MAO activity in all tissue preparations. This pattern is similar to that found for mitochondrial enzymes and antioxidant metabolism (Sections 9.3.3.2 and 9.3.3.3).

The cytochrome P-450-dependent enzymes have been studied because of their function in drug and carcinogen metabolism. Palmer et al. (1971, 1972) found that hamsters exposed to 1,470 $\mu g/m^3$ (0.75 ppm) of O_3 for 3 hr had lower benzo(a)pyrene hydroxylase activity in the lung. Goldstein et al. (1975) showed that rabbits exposed to 1,960 $\mu g/m^3$ (1 ppm) of O_3 for 90 min had decreased levels of lung cytochrome P-450. Maximal decreases occurred 3.6 days following exposure. Recovery to control values occurred somewhere between 8 days and 45 days. Cytochrome P-450-mediated activity of benzphetamine N-demethylase in the lung was lowered by a 24-hr exposure to 1,960 $\mu g/m^3$ (1 ppm) of O_3 in rats (Montgomery and Niewoehner, 1979). The cytochrome P-450 dependent activity began to recover by 4 days postexposure but was still decreased. Complete recovery occurred by 1 week. Cytochrome b_5-mediated lipid desaturation was stimulated by O_3 4, 7, and 14 days postexposure. Immediately after exposure, the desaturase activity was quite depressed, but this was attributed to anorexia in the rats, and not to O_3. Cytochrome P-450-dependent enzymes exist in multiple forms, because they have different substrate affinities that overlap. Measuring activity with only one substrate does not characterize a

TABLE 9-6. MONOOXYGENASES

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
μg/m³	ppm					
392 980 1568	0.2 0.5 0.8	MAST NBKI	Continuous or 8 hr/day for 7 days	Concentration-related linear increase in NADPH cytochrome c reductase activity. No difference between continuous and intermittent.	Rat	Mustafa and Lee, 1976; Schwartz et al., 1976; Mustafa et al., 1977
1470 5880 19,600	0.75 3.0 10.0	I	3 hr	Decreased activity of benzpyrene hydroxylase in lung parenchyma.	Hamster	Palmer et al., 1971
1470 5880 19,600	0.75 3.0 10.0	I	3 hr	Decreased activity of benzpyrene hydroxylase in tracheobronchial mucosae.	Rabbit	Palmer et al., 1972
1568	0.8	ND	Continuous for 7 days	High O_3 level reduced monoamine oxidase activity; low O_3 level increased it.	Rat	Mustafa et al., 1977
3920	2.0		8 hr			
1568	0.8	I	Continuous for 7 days	High O_3 level decreased activity of NADPH cytochrome c reductase; low level increased it.	Rat	DeLucia et al., 1972, 1975a
3920	2.0		8 hr			
392 1568 1568	0.2 0.5 0.8	I	Continuous or 8 hr/day	Increased activity of NADPH cytochrome c reductase. At 0.8 ppm, increase began at day 2 of exposure.	Rat	DeLucia et al., 1975a
1568	0.8		8 hr/day	No change in NADPH cytochrome c reductase activity.	Monkey	
1568	0.8	I	Continuous for 7 days	Increased activity of NADPH cytochrome c reductase on days 2 through 7. Maximal increase on day 4.	Rat	Mustafa and Lee, 1976

TABLE 9-6. MONOOXYGENASES (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
μg/m³	ppm					
1960	1.0	ND	90 min	Decreased levels of lung cytochrome P-450. Maximal decrease at 3.6 days postexposure.	Rabbit	Goldstein et al., 1975
1960	1.0	MAST	24 hr	50% decrease in benzphetamine N-demethylase activity 1 day postexposure; return to control levels by 1 wk postexposure. Stimulation of cytochrome b_5-mediated lipid desaturation.	Rat	Montgomery and Niewoehner, 1979
5880	3	NBKI	10 min before lung perfusion and continuous throughout experiment.	Decreased enzymatic conversion of arachidonic acid to prostaglandins when using isolated ventilated perfused lung.	Rat	Menzel et al., 1976

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas solid chemiluminescence; NBKI = neutral buffered potassium iodide; I = Iodometric; ND = not described.

[b] Calibration method: UKI = unbuffered potassium iodide.

single enzyme. More importantly, the relatively long time for recovery suggests that cell injury, rather than enzyme destruction, has occurred. Benzo(a)pyrene hydroxylase is the first major enzyme in the activation of benzo(a)pyrene and several other polycyclic hydrocarbons to an active carcinogen. However, additional enzymes not studied after O_3 exposure are involved in the activation, which makes full interpretation of the effect of O_3 on this metabolism impossible. The impact of the decrease in cytochrome P-450 depends on the activation or detoxification of the metabolized compound by this system.

Also involved in mixed function oxidase metabolism is NADPH cytochrome c reductase. As with other classes of enzymes (Sections 9.3.3.2; 9.3.3.3), acute exposure to a high O_3 level (3920 $\mu g/m^3$, 2 ppm; 8 hr) reduced NADPH cytochrome c reductase activity (DeLucia et al., 1972, 1975a). After a continuous or 8 hr/day exposure of rats for 7 days, the activity of NADPH cytochrome c reductase increased linearly in a concentration-related fashion (392, 980, and 1568 $\mu g/m^3$; 0.2, 0.5, and 0.8 ppm) (Mustafa and Lee, 1976; Schwartz et al., 1976; Mustafa et al., 1977). Continuous and intermittent exposures were not different. The time course of the response to 1568 $\mu g/m^3$ (0.8 ppm) was an increase in activity that began at day 2, peaked at day 4, and was still increased at day 7 of continuous exposure in the rat (Mustafa and Lee, 1976). The rat, but not the Rhesus monkey, is apparently affected after exposure for 8 hr/day for 7 days to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 (DeLucia et al., 1975a). However, monkey data were not reported in any detail.

9.3.3.5 Lactate Dehydrogenase and Lysosomal Enzymes. Lactate dehydrogenase (LDH) and lysosomal enzymes are frequently used as markers of cellular damage if levels are observed to increase in lung lavage or serum/plasma, because these enzymes are released by cells upon certain types of damage. Effects of O_3 on these enzymes are described in Table 9-7. No lung lavage studies have been reported; whole-lung homogenates were used. Therefore, it is not possible to determine whether the observed increases reflect a leakage into lung fluids and a compensatory resynthesis in tissue or cellular changes (See Section 9.3.1), such as an increase in type 2 cells and alveolar macrophages and polymorphonuclear leukocytes rich in lysosomal hydrolases. In some instances, correlation with plasma values was sought. They are described briefly here; more detail is given in Section 9.4.3.

Lactate dehydrogenase is an intracellular enzyme that consists of two subunits combined as a tetramer. Various combinations of the two basic subunits change the electrophoretic pattern of LDH so that its various isoenzymes can

TABLE 9-7. LACTATE DEHYDROGENASE AND LYSOSOMAL ENZYMES

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
μg/m³	ppm					
196	0.1	NBKI	Continuous for 7 days	Increase in total LDH activity in diet group receiving 0 ppm vitamin E. Groups with 11 or 110 ppm of vitamin E had no effect.	Rat	Chow et al., 1981
392 980 1568	0.2 0.5 0.8	MAST, NBKI	Continuous for 8 days or 8 hr/days for 7 days	Increased lung lysozyme activity only after continuous exposure to 0.8 ppm.	Rat	Chow et al., 1974
980 1568	0.5 0.8	NBKI	8 hr/day for 7 days	Increased LDH activity in lungs. Change in LDH isoenzyme distribution at 0.8 ppm.	Rat	Chow et al., 1977
1568	0.8			No change in total LDH activity or isoenzyme pattern in lungs.	Monkey	
1372	0.7	NBKI	Continuous for 5 days	Specific activities of various lysosomal hydrolases increased.	Rat	Dillard et al., 1972
1568	0.8		Continuous for 7 days			
1372- 1568	0.7- 0.8	MAST, NBKI	Continuous for 7 days	Increase in lung acid phosphatase activity; no observed increases in B-glucuronidase activity.	Rat	Castleman et al., 1973a
1568	0.8	MAST, NBKI	Continuous for 7 days	Bronchiolar epithelium had decreased NADH and NADPH activities and increased ATPase activity.	Rat	Castleman et al., 1973b
1568	0.8	MAST, NBKI	Continuous for 7 days	Increase in LDH activity not affected by vitamin E (0 or 45 mg/kg diet).	Rat	Chow and Tappel, 1973

[a] Measurement method: MAST = KI-coulometric (Mast meter); NBKI = neutral buffered potassium iodide.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

be detected. Chow and Tappel (1973) found an increase in LDH activity in the homogenate of rat lungs exposed to 1568 μg/m^3 (0.8 ppm) of O_3 continuously for 7 days. Lower levels (196 μg/m^3, 0.1 ppm; 7 days continuous) only increased LDH activity of lung homogenate when rats were on a diet deficient in vitamin E (Chow et al., 1981). Vitamin E levels in the diet did not significantly influence the response. In following up this finding, Chow et al. (1977) studied LDH activity and isoenzyme pattern (relative ratios of different LDH isoenzymes) in the lungs, plasma, and erythrocytes of O_3-exposed rats (980 or 1,568 μg/m^3, 0.5 or 0.8 ppm) and monkeys (1568 μg/m^3, 0.8 ppm). Exposure was for 8 hr/day for 7 days. In monkeys, no significant changes in either total LDH activity or isoenzyme pattern in lungs, plasma, or erythrocytes were detected. The total LDH activity in the lungs of rats was increased after exposure to 1,568 or 980 μg/m^3 (0.8 or 0.5 ppm), but no changes in the plasma or erythrocytes were detected. The isoenzyme pattern of LDH following O_3 exposure was more complex, with the LDH-5 fraction significantly decreased in lungs and plasma of rats exposed to 1,568 μg/m^3 (0.8 ppm). The LDH-4 fraction in lungs and plasma and the LDH-3 fraction in lungs were increased. No changes were discernible in rats exposed to 980 μg/m^3 (0.5 ppm) of O_3. The changes in LDH isoenzyme pattern appeared to be due to a relative increase in the LDH isoenzymes containing the H (heart type) subunits. Although the increase in LDH suggests cytotoxicity after O_3 exposure, no clear-cut interpretation can be placed on the importance of the isoenzyme pattern. Some specific cell types in the lung may contain more H-type LDH than others and be damaged by O_3 exposure. Further studies of the fundamental distribution of LDH in lung cell types are needed to clarify this point.

Lysosomal enzymes have been found to increase in the lungs of animals exposed to O_3 at concentrations of 1,372 ± 294 μg/m^3 (0.70 ± 0.15 ppm) for 5 days and 1,548 ± 274 μg/m^3 (0.79 ± 0.14 ppm) for 7 days, whether detected by biochemical (whole-lung homogenates and fractions) or histochemical means (Dillard et al., 1972). Dietary vitamin E (0 to 1500 mg/kg diet) did not influence the effects. These increased activities were attributed to the infiltration of the lung by phagocytic cells during the inflammatory response phase from O_3 exposure. Similarly, Castleman et al. (1973a,b) found that activity of lung acid phosphatase was increased in young rats that had been exposed to 1,372 to 1,568 μg/m^3 (0.7 to 0.8 ppm) of O_3 continuously for 7 days. Increases in β-glucuronidase activity were not observed. The histochemical and cytochemical localization suggested that O_3 exposure results in damage to the lung's lysosomal membranes. Castleman et al. (1973b) also found that the bronchiolar epithelium in infiltrated areas had lower NADPH- and NADH diaphorase activities and higher ATPase activities than similar epithelium of control lungs. They discussed in greater detail the enzymatic distribution within the lung and suggested that some of the pyridine nucleotide-dependent reactions could represent an enzymatic protective mechanism operating locally in the centriacinar regions of O_3-exposed lungs. Chow et al. (1974) also observed an increase in lysozyme activity (lung homogenate) in rats exposed continuously to 1568 $\mu g/m^3$ (0.8 ppm) for 8 days but not in rats exposed intermittently (8 hr/day, 7 days). No effect was seen in continuous or intermittent exposure of rats to 392 or 980 $\mu g/m^3$ (0.2 or 0.5 ppm) of O_3.

Lysosomal acid hydrolases include enzymes that digest protein and can initiate emphysema. The contribution of the increases in these enzymes observed by some after O_3 exposure to morphological changes has not been demonstrated.

9.3.3.6 Protein Synthesis. The effects of O_3 on protein synthesis can be divided into two general areas: (1) the effects on the synthesis of collagen and related structural connective tissue proteins, and (2) the effects on the synthesis or secretion of mucus. The studies are summarized in Table 9-8.

Hesterberg and Last (1981) found that increased collagen synthesis caused by continuous exposure to O_3 at 1568, 2352, and 2940 $\mu g/m^3$ (0.8, 1.2, and 1.5 ppm) for 7 days could be inhibited by concurrent treatment with methylprednisolone (1 to 50 mg/kg/day).

Hussain et al. (1976a,b) showed that lung prolyl hydroxylase activity and hydroxyproline content increased on exposure of rats to 980 and 1568 $\mu g/m^3$ (0.5 and 0.8 ppm) of O_3 for 7 days. At 392 $\mu g/m^3$ (0.2 ppm), O_3 produced a statistically insignificant increase in prolyl hydroxylase activity. Prolyl hydroxylase is the enzyme that catalyzes the conversion of proline to hydroxyproline in collagen. This conversion is essential for collagen to form the fibrous conformation necessary for its structural function. Hydroxyproline is an indirect measure of collagen content. When rats were exposed to 980 $\mu g/m^3$ (0.5 ppm) of O_3 for 30 days, the augmentation of activity seen earlier at 7 days of exposure had diminished, and by 60 days, the enzyme activity was within the normal range despite continued O_3 exposure. When rats were exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3, the prolyl hydroxylase activity continued to rise for about 7 days; hydroxyproline content of the lung rose to a maximum value at about 3 days after exposure began and remained equivalently elevated

TABLE 9-8. EFFECTS OF OZONE ON LUNG PROTEIN SYNTHESIS

Ozone concentration $\mu g/m^3$	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
392 784 1176	0.2 0.4 0.6	ND	8 hr/day for 3 days	Decreased rate of glycoprotein secretion by tracheal explants at 0.6 ppm.	Rat	Last and Kaizu, 1980; Last and Cross, 1978
1568	0.8		Continuous for 1 through 90 days	Decreased rate of glycoprotein secretion.		
980	0.5		Continuous for 3 or 14 days and combined with H_2SO_4	Increased rate of glycoprotein secretion.		
392 980 1568	0.2 0.5 0.8	MAST	Continuous for 7 days	Concentration-dependent increase in lung prolyl hydroxylase activity. No effect at 0.2 ppm. Metabolic adaptation suggested at 980 $\mu g/m^3$ (0.5 ppm) At 0.8 ppm, collagen and noncollagenous protein synthesis increased; effect on prolyl hydroxylase returned to normal by about 10 days postexposure, but hydroxyproline was still increased at 28 days.	Rat	Hussain et al., 1976a,b
392 784 1176 1568	0.2 0.4 0.6 0.8	UV	8 hr/day for 1 to 90 days	At 0.8 ppm, tracheal explants had decreased rate of glycoprotein secretion for up to 1 wk, followed by increased rate up to 12 wks. Three day exposure to three lower concentrations caused decrease at only 0.6 ppm.	Rat	Last et al., 1977
392 1568 3920	0.2 0.8 2.0	UV, NBKI	6 hr/day, 5 days/ wk, 12.4 wk (62 days of exposure)	Decrease in collagen and elastin at 0.2 and and 0.8 ppm; increase at 2 ppm.	Rat	Costa et al., 1983
882	0.45	ND	Continuous for 7 days	Increased collagen synthesis at 5 and 7, but not 2 days of exposure. Similar pattern for increase in superoxide dismutase activity. Increased prolyl hydroxylase activity at 2, 3, 5, and 7 days of exposure; maximal effect at day 5.	Mouse	Bhatnagar et al., 1983
1568	0.8	ND	Continuous for 90 days	Increase in prolyl hydroxylase activity through 7 days. No effect 20 days and beyond.	Rat	
980-3920	0.5-2	UV	1, 2, or 3 wk	Increased rate of collagen synthesis; fibrosis of alveolar duct walls; linear concentration response.	Rat	Last et al., 1979

TABLE 9-8. EFFECTS OF OZONE ON LUNG PROTEIN SYNTHESIS (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
980	0.5	UV	Continuous for up to 180 days	Increase in protein and hydroxyproline content of lungs. No change 2 mo postexposure.	Rat	Last and Greenberg, 1980
980- 2940	0.5- 1.5	NBKI	Continuous for 7 days	O_3 caused linear, concentration-related increases in collagen synthesis; $(NH_4)_2SO_4$ combined with O_3 increased collagen synthesis rates by 180% at 1.2 and 1.5 ppm O_3.	Rat	Last et al., 1983
5000 $(NH_4)_2SO_4$						
980	0.5	NBKI	Continuous for 3 to 50 days	O_3 increased collagen synthesis at 3, 30, and 50 days; H_2SO_4 combined with O_3 increased collagen synthesis rates by 220%.		
1000 H_2SO_4						
1254	0.64	UV	Continuous for 3 days	No significant effect of O_3 or $(NH_4)_2SO_4$ on collagen synthesis; $(NH_4)_2$ SO_4 + O_3 increased collagen synthesis rates by 230%.	Rat	Last et al., 1984a
5000 $(NH_4)_2SO_4$						
1882	0.96	UV	Continuous for 7 to 14 days	Interstitial edema and inflammation of proximal alveolar ducts; $(NH_4)_2$ SO_4 increased the severity of O_3 effects at lesion sites without increasing the number of lesions.		
5000 $(NH_4)_2SO_4$						
1254	0.64	UV	8 hr/day, 361 days	Increase in collagen content	Monkey	Last et al., 1984b
1254 1882	0.64 0.96	UV	Continuous for 90 days; intermittent units of 5 days (8 hr/day) of O_3, and 9 days of air, repeated 7 times with a total of 35 exposure days over a 90-day interval	Equivalent increase in collagen in all but the 0.64 ppm continuous group which only had a marginal ($p < 0.1$) increase.	Rat (young adult)	Last et al., 1984b

TABLE 9-8. EFFECTS OF OZONE ON LUNG PROTEIN SYNTHESIS (continued)

Ozone concentration μg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
1254 1882	.64 .96		8 hr day 7 days/wk, 6 wk	Increased collagen content at 0.96 ppm O_3. At 6 wk post-exposure, both O_3 levels increased collagen. Suggestion of progressive effects.	Rat (weanling)	
1882	0.96		Continuous for 3 wk	Both groups had an equivalent increase in lung collagen content.	Rat, (young adult)	
2352	1.2		Continuous for 1 wk, then 2 wk of air			
1882	.96		Intermittent units of 3 days (8 hr/day) of O_3, and 4 days of air, repeated 6 times	No effect on collagen content.	Rat, (weanling)	
1882	.96		7 wk, 8 hr/day, 7 days/wk	Increase in collagen content.	Rat, (young adult)	
1254	0.64	UV	Continuous for 7 days	Increased lung collagen and protein synthesis rates; results of statistical analyses were not reported.	Rat	Myers et al., 1984
1568	0.8	ND	Continuous for 7 days	Decreased protein synthesis on day 1; increased synthesis day 2 and thereafter; peak response on days 3 and 4.	Rat	Mustafa et al., 1977
1568	0.8	NBKI	Continuous for 3 days	Increased protein synthesis; recovery by 6 days later; after re-exposure 6, 13, or 27 days later, protein synthesis increased.	Rat	Chow et al., 1976b
1568 2352 2940	0.8 1.2 1.5	UV	Continuous for 7 days	Net rate of collagen synthesis by lung minces increased in concentration-dependent manner; methyl prednisolone administered during O_3 exposure prevented increase.	Rat	Hesterberg and Last, 1981

[a] Measurement method: MAST = KI-coulometric (Mast meter); UV = UV photometry; NBKI = neutral buffered potassium iodide; ND = not described.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

through day 7 of exposure. Incorporation of radiolabeled amino acids into collagen and noncollagenous protein rose to a plateau value at about 3 (collagenous) or 4 to 7 (noncollagenous) days after exposure. Synthesis of collagen was about 1.6 times greater than that of noncollagenous proteins during the first few days of exposure; no major differences were apparent by 7 days of exposure. After the 7-day exposure ended, about 10 days were required for recovery to initial values of prolyl hydroxylase. However, hydroxyproline levels were still increased 28 days postexposure. This suggests that although collagen biosynthesis returns to normal, the product of that increased synthesis, collagen, remains stable for some time.

The shape of the concentration-response curve was investigated by Last et al. (1979) for biochemical and histological responses of rat lungs after exposure to ozone for 1, 2, or 3 weeks at levels ranging from 980 to 3920 $\mu g/m^3$ (0.5 to 2 ppm). A general correlation was found between fibrosis detected histologically and the quantitative changes in collagen synthesis in minces of O_3-exposed rat lungs. The stimulation of collagen biosynthesis was essentially the same, regardless of whether the rats had been exposed for 1, 2 or 3 weeks; it was linearly related to the O_3 concentration to which the rats were exposed.

Protein deficiency and food restriction do not have a major influence on the effects of O_3 on lung hydroxyproline, lung elastin, or apparent rates for lung collagen synthesis and elastin accumulation (Myers et al., 1984). In this study weanling or young adult rats were exposed continuously to 1254 $\mu g/m^3$ (0.64 ppm) O_3 for 7 days. It appears that O_3 caused an increase in apparent lung collagen and protein synthetic rates and no major change in elastin accumulation, but the results of statistical analyses were not reported.

Continuous exposure of mice for 7 days to 882 $\mu g/m^3$ (0.45 ppm) of O_3 caused an increase in collagen synthesis after 5 or 7, but not 2 days of exposure (Bhatnagar et al., 1983). The 5- and 7-day results showed little if any difference. The effect on synthesis of noncollagen protein was not significant. Prolyl hydroxylase activity was also increased at 2, 3, 5, and 7 days of exposure, with the maximal increase at day 5. The day 7 results were only slightly different (no statistical analysis) from the day 2 data. Superoxide dismutase activity was investigated, because it has been observed (in other studies) to prevent a superoxide-induced increase in collagen synthesis and prolyl hydroxylase. Activity of superoxide dismutase increased in a pattern parallel to that for collagen synthesis.

Bhatnagar et al. (1983) also studied rats exposed continuously for 90 days to 1568 μg/m^3 (0.8 ppm) of O_3. Prolyl hydroxylase activity continued to increase through 7 days of exposure. By 20, 50, and 90 days of exposure, no significant effects were observed.

Last and his colleagues performed a series of studies to evaluate the effects of subchronic exposure to O_3 on rats. In one experiment (Last et al., 1984b), young adult (60-65 days old) rats were exposed continuously for 90 days to 1254 μg/m^3 (0.64 ppm) or 1882 μg/m^3 (0.96 ppm) O_3; the higher level increased lung collagen content, while the lower level only caused a marginal ($p < 0.1$) increase. Rats were exposed to these same concentrations in an intermittent regimen consisting of 5 days of exposure (8 hr/day), 9 days of air, 5 days of exposure, 9 days of air, etc., for a total of 35 days of O_3 and 54 days of air within the 90-day experiment. Both concentrations of O_3 caused an equivalent increase in collagen content. The magnitudes of the effects after continuous or intermittent exposure were not statistically different.

Weanling rats (28 days old) were examined after a 6-wk exposure (8 hr/day, 7 days/wk) to 1254 μg/m^3 (0.64 ppm) or 1882 μg/m^3 (0.96 ppm) O_3 (Last et al., 1984b). Immediately after exposure, only those animals exposed to 1882 μg/m^3 (0.96 ppm) O_3 exhibited an increase in lung collagen content. However, 6 wk postexposure, animals exposed to both the high and low concentrations of O_3 had increases in collagen content. It appears that the collagen content increased during this postexposure period, but no statistical comparisons were reported.

Young adult rats exposed continuously for 3 weeks to 1882 μg/m^3 (0.96 ppm) O_3 had an increase in lung collagen (Last et al., 1984b). Rats exposed to 2352 μg/m^3 (1.2 ppm) O_3 for 1 wk and examined 2 wk later had an increase in collagen equivalent to that of the 3-wk exposure group (1882 μg/m^3, 0.96 ppm).

Weanling rats were also studied after intermittent exposure to 1882 μg/m^3 (0.96 ppm) O_3 (Last et al., 1984b). The regimen was 3 days of exposure for 8 hr/day, followed by 4 days of air; this unit was repeated 6 times. No significant changes in collagen content occurred.

Lung collagen of juvenile cynomolgus monkeys (6-7 mo old at start of exposure), exposed to 1254 μg/m^3 (0.64 ppm) O_3 for 8 hr/day, 7 days/wk for 1 yr (361 days), was studied by Last et al. (1984b). Collagen content was increased. Collagen type ratios were determined, and there were no apparent shifts in collagen types; however, the authors report that given the surgical variation, small shifts in collagen types would not be likely to be detected.

Rats were exposed to 980 $\mu g/m^3$ (0.5 ppm) continuously for up to 180 days and examined at various times during exposure and 2 months after exposure ceased (Last and Greenberg, 1980). The total protein content of the lungs increased during exposure, with the greatest increase occurring after 88 days of exposure. By 53 days postexposure, values had returned to control levels. Hydroxyproline content of the lungs also increased following 3, 30, 50, or 88, but not 180, days of exposure. No such effect was observed at the 53-day postexposure examination. The rates of protein and hydroxyproline synthesis were also measured. Protein synthesis was not affected significantly. Although the authors mentioned that hydroxyproline synthesis rates "appeared to be greater," statistical significance was not discussed and values appeared to be only slightly increased, considering the variability of the data. In discussing their data, the authors referred to a concurrent morphological study (Moore and Schwartz, 1981) that showed an increase in lung volume, mild thickening of the interalveolar septa and alveolar interstitium, and an increase in collagen (histochemistry) in these areas. Different results for collagen levels were observed after another longer-term exposure (6 hr/day, 5 days/wk, 12.4 wk) to 392, 1568, or 3920 $\mu g/m^3$ (0.2, 0.8, or 2.0 ppm) of O_3 (Costa et al., 1983). At the two lower concentrations, rats exhibited an equivalent decrease in hydroxyproline. At the highest concentration, an increase was observed. Similar findings were made for elastin levels.

Most reports, such as those described above, are on the effects of O_3 on collagen synthesis. Very little is known about the effects of O_3 on collagen turnover (i.e., the integration of synthesis and degradation). Curran et al. (1984) found that in vitro exposure to high levels of O_3 (19600 $\mu g/m^3$, 10 ppm for 1-4 hr) caused degradation of collagen. A lower level, 490 $\mu g/m^3$ (0.25 ppm) did not cause degradation, but the collagen became more susceptible to proteolytic degradation.

Last (1983) and Last et al. (1983) investigated the interaction between O_3 and aerosols of ammonium sulfate [$(NH_4)_2SO_4$] and sulfuric acid (H_2SO_4) in rats. Rats were exposed continuously for 7 days to four concentrations of O_3 ranging from 980 $\mu g/m^3$ to 2940 $\mu g/m^3$ (0.5 to 1.5 ppm) O_3. According to the authors, these levels were determined by the KI method and should be multiplied by 0.8 to compare to UV photometric methods (i.e., 0.4 to 1.2 ppm). The authors' actual measured values are noted here. The O_3 exposure resulted in a linear, concentration-related increase in collagen synthesis rate. A 7-day continuous exposure to 5 mg/m^3 $(NH_4)_2SO_4$ (0.8-1.0 µm mass median aerodynamic diameter, MMAD) had no effect. However, when mixtures of O_3 from 1568 to 2940 µg/m^3 (0.8 to 1.2 ppm) and 5 mg/m^3 $(NH_4)_2SO_4$ were used, the collagen synthesis rate increased to about 180% over the rates of O_3 for the higher O_3 levels (apparently 2352 and 2940 µg/m^3, 1.2 and 1.5 ppm).

Mixtures of O_3 and H_2SO_4 were also reported (Last et al., 1983). Rats were exposed to 980 µg/m^3 (0.5 ppm) O_3 or this O_3 level in combination with 1000 µg/m^3 H_2SO_4 (0.38 µm, MMAD) continuously for 3 to 50 days. As expected, O_3 exposure increased collagen synthesis rates at the three times of examination (3, 30, and 50 days). The mixture of O_3 and H_2SO_4 caused greater effects. Examination of the slope ratio of regression lines indicated that H_2SO_4 in the mixture caused a 220 percent enhancement. The authors state that H_2SO_4 alone had no effects.

The statistical procedures applied in these collagen studies (Last et al., 1983) were questioned by Krupnick and Frank (1984) in a letter to the editor. The general criticism was that too few statistical tests were applied to test the hypothesis of synergism and that too few pollutant data points were used in the design to develop robust regression lines. The authors (Last et al. 1983) responded that they did apply most of the statistical tests and found significant differences between regressions comparing O_3 only to O_3 plus aerosols, but the journal would not accede to publishing these analyses.

The proximal acinar regions of rats from the above-mentioned collagen studies, exposed for 7 days to 2352 µg/m^3 (1.2 ppm) O_3 alone or in combination with 5000 µg/m^3 $(NH_4)_2SO_4$, were also evaluated. Ammonium sulfate alone caused no morphological or morphometric effects. Ozone exposure resulted in a thickening of the interstitium and an influx of inflammatory cells. The mixture of O_3 and $(NH_4)_2SO_4$ caused the same response plus an apparent deposition of fibrous material. These lesions were then examined morphometrically. In the O_3 and O_3 plus $(NH_4)_2SO_4$ groups, there were no changes in the volume ratio of the lesion per lung or the volume ratio of the extracellular connective tissue per lesion. In examining volume density of different cell types in the lung lesions, both groups had an increased percentage of fibroblasts and smooth muscle. When the number of cells of each type present per area of lesion was calculated, there was a 340 percent increase in the number of fibroblasts in the O_3 plus $(NH_4)_2SO_4$ group compared to the O_3 group. These findings correlate well with the biochemical effects.

These studies were expanded by Last et al. (1984a) to better evaluate the influence of length of exposure. All exposures were continuous to O_3, $(NH_4)_2SO_4$, or a mixture of the two; the ammonium sulfate concentration was about 5000 μg/m^3 (0.5 μm MMAD), the precise concentration depending upon the experiment. After 3 days of exposure to either 1254 μg/m^3 (0.64 ppm) O_3 or $(NH_4)_2SO_4$, no significant effects on collagen synthesis of rats were observed; the mixture of O_3 and $(NH_4)_2SO_4$ more than doubled the collagen synthesis rate over controls. The pulmonary lesions (i.e., aggregations of interstitial inflammatory cells in terminal bronchioles) of the proximal acinus in these rats were examined histologically and morphometrically. Ammonium sulfate alone caused no effects. In the O_3 and O_3 plus $(NH_4)_2SO_4$ groups, there was no fibrosis, but thickening by edema and inflammatory cell infiltrate was observed. Morphometrically, $(NH_4)_2SO_4$ caused no effects. However, O_3 and O_3 plus $(NH_4)_2SO_4$ increased total cell numbers in lesions, with the mixture producing a significantly greater increase over O_3 alone. This increase was principally due to an increase in the numbers of macrophages, monocytes, and fibroblasts. The increase in the number of fibroblasts is consistent with the biochemical findings.

Similar measurements were made on rats exposed continuously for 7 days to 1882 μg/m^3 (0.96 ppm) O_3, 5000 μg/m^3 $(NH_4)_2SO_4$, or a mixture of the two (Last et al., 1984a). Control data were not presented and all statistical comparisons reported were in reference to the O_3 alone group. Ammonium sulfate exposure resulted in fewer numbers of cells than O_3 alone. For macrophage and monocyte numbers, O_3 and $(NH_4)_2SO_4$ were apparently additive. For fibroblast and total cell numbers, O_3 and $(NH_4)_2SO_4$ were apparently synergistic.

The frequency of occurrence of lesions was also examined in the 3-day and the 7-day studies described above (Last et al., 1984a). There was no difference in the frequency of lesion occurrence between O_3 and O_3 plus $(NH_4)_2SO_4$. The individual lesions were larger in the O_3 plus $(NH_4)_2SO_4$ group; the $(NH_4)_2SO_4$ group had almost no lesions. Using collagen staining procedures, the O_3 plus $(NH_4)_2SO_4$ group had a greater volume of collagen than did lesions in the O_3 alone group. The authors summarized these studies (Last et al., 1983; Last et al., 1984a) by stating that $(NH_4)_2SO_4$ increases the severity of O_3 effects at lesion sites without increasing the number of lesions; and the cellular changes correlate with biochemical and histological indications of potential later fibrosis. From all these studies it is apparent that synergism occurs. However, it further appears that the synergism is dependent on the concentration of O_3. For instance, Last et al. (1983) demonstrated that 5000 μg/m^3 $(NH_4)_2SO_4$

increased collagen synthesis rates in rats exposed to 2352 μg/m^3 or 2940 μg/m^3 (1.2 or 1.5 ppm) O_3, but not in those exposed to 1568 μg/m^3 (0.8 ppm) O_3.

Protein synthesis (incorporation of radiolabeled leucine) was increased in rats after 3 days of continuous exposure to 1568 μg/m^3 (0.8 ppm) of O_3 (Chow et al., 1976b). Recovery had occurred by 6 days postexposure. No adaptation was observed, because when animals were re-exposed to the same O_3 regimen 6, 13 or 27 days after the first exposure, protein synthesis increased as it had earlier. Mustafa et al. (1977) investigated the time course of the increased in vivo incorporation of radioactive amino acids. Rats were exposed continuously for 7 days to 1568 μg/m^3 (0.8 ppm) of O_3. No statistics were reported. One day of exposure caused a decrease in protein synthesis. However, by day 2, an increase occurred, which peaked on days 3 and 4 of exposure. On day 7, the effect had not diminished. The authors attributed this finding to synthesis of noncollagenous protein. They also found no radioactive incorporation into blood or alveolar macrophages. Hence, the observed increases were due to lung tissue protein synthesis, and not a concurrent influx of alveolar macrophages or serum into the lungs.

The production of mucous glycoproteins and their secretion by tracheal explants have been reviewed by Last and Kaizu (1980). Mucous glycoprotein synthesis and secretion were measured by the rate of incorporation of ^3H-glucosamine into mucous glycoproteins and their subsequent secretion into supernatant fluid of the tracheal explant culture medium. This method has been found to be a reproducible index of mucous production, and these authors maintain that this measurement ex vivo following exposure in vivo is representative of injuries occurring in vivo. When rats were exposed to 1568 μg/m^3 (0.8 ppm) of O_3 for 8 hr/day for 1 to 90 days, Last et al. (1977) found a depression of glycoprotein synthesis and secretion into the tissue culture medium for the initial week that was statistically significant only on days 1 and 2 of exposure. Rebound occurred subsequently, with increased glycoprotein secretion for at least 12 weeks of continued exposure to O_3 (only significant at 1 and 3 months of exposure). Rats were also exposed intermittently for 3 days to 1176, 784, and 392 μg/m^3 (0.6, 0.4, 0.2 ppm) of O_3. Glycoprotein secretion decreased only at the higher concentration. Tracheal explants from Bonnet monkeys exposed to 0, 980, or 1568 μg/m^3 (0, 0.5 or 0.8 ppm) O_3 for 7 days appeared to have increased rates of secretion of mucus (Last and Kaizu, 1980). However, few monkeys were used and statistical analysis was not reported.

A combination exposure to O_3 (980 μg/m^3; 0.5 ppm) and sulfuric acid (H_2SO_4) aerosol (1.1 mg/m^3) caused complex effects on mucous secretions in rats (Last and Kaizu, 1980; Last and Cross, 1978). A 3-day exposure to 980 μg/m^3 (0.5 ppm) of O_3 decreased mucous secretion rates, but H_2SO_4 had no effect. In rats exposed to the combination of H_2SO_4 aerosol and O_3, mucous secretion significantly increased. After 14 days of continuous exposure, the rats receiving a combined exposure to both H_2SO_4 aerosol and O_3 had elevated values (132 percent) over the control group of animals. Because mucous secretion and synthesis are intimately involved in diminishing the exposure of underlying cells to O_3 and removing adventitiously inhaled particles, alterations in the mucous secretory rate may have significant biological importance. Experiments reported to date do not clearly indicate what human health effects may be likely, nor their importance.

9.3.3.7 Lipid Metabolism and Content of the Lung. If O_3 initiates peroxidation of unsaturated fatty acids in the lung, then changes in the fatty acid composition of the lung indicative of this process should be detectable. Because the fatty acid content of the lung depends on the dietary intake, changes in fatty acid content due to O_3 exposure are difficult to determine in the absence of rigid dietary control. Studies on lipid metabolism and content of the lung are summarized in Table 9-9. Generally, the unsaturated fatty acid content decreased in rats exposed to 980 μg/m^3 (0.5 ppm) of O_3 for up to 6 weeks (Roehm et al., 1972).

Peroxidation of polyunsaturated fatty acids produces pentane and ethane to be exhaled in the breath of animals (Donovan and Menzel, 1978; Downey et al., 1978). A discussion of the use of ethane and pentane as indicators of peroxidation is presented by Gelmont et al. (1981) and Filser et al. (1983). Normal animals and humans exhale both pentane and ethane in the breath. Dumelin et al. (1978b) found that exhalation of ethane decreased and exhalation of pentane increased when rats were deficient in vitamin E and exposed to 1960 μg/m^3 (1 ppm) of O_3 for 60 min. The provision of 11 (minimum vitamin requirement) or 40 IU (supplemented level) of vitamin E acetate per kg of diet resulted in a decrease in expired ethane and pentane after O_3 exposure. Dumelin et al. (1978a) also measured breath ethane and pentane in Bonnet monkeys exposed to 0, 980, or 1568 μg/m^3 (0, 0.5, or 0.8 ppm) of O_3 for 7, 28, or 90 days. They failed to detect any additional ethane or pentane in the breath of these monkeys and attributed the lack of such additional evolution to be due to the high level of vitamin E provided in the food.

TABLE 9-9. EFFECTS OF OZONE EXPOSURE ON LIPID METABOLISM AND CONTENT OF THE LUNG

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
μg/m³	ppm					
980	0.5	I	Continuous for 2, 4, or 6 wk	Increase in arachidonic and palmitic acids; decrease in oleic and linoleic acids.	Rat	Roehm et al., 1972
980 1568	0.5 0.8	UV	8 hr/day for 7, 28, or 90 days	No effect on ethane and pentane production in animals fed diets supplemented with high levels of vitamin E.	Monkey	Dumelin et al., 1978a
1960	1.0	NBKI	60 min	With vitamin E-deficient diet, increased pentane production and decreased ethane production. With vitamin E supplementation of 11 or 40 IU vitamin E/kg diet, decreased ethane and pentane production.	Rat	Dumelin et al., 1978b
1960	1.0	ND	4 hr	Decreased incorporation of fatty acids into lecithin.	Rabbit	Kyei-Aboagye et al., 1973

[a] Measurement method: NBKI = neutral buffered potassium iodide; UV = UV photometry; I = iodometric; ND = not described.

Kyei-Aboagye et al. (1973) found that the synthesis of lung surfactant in rabbits, as measured by dipalmitoyl lecithin synthesis, was inhibited by exposure to 1960 μg/m^3 (1 ppm) of O_3 for 4 hr. Pulmonary lavage showed an increase in radiolabeled lecithins. The authors proposed that O_3 may decrease lecithin formation while simultaneously stimulating the release of surfactant lecithins. This may suggest the presence of a larger disarrangement of lipid metabolism following O_3 exposure. However, although changes in lipid composition of lavage fluid occur, the changes apparently do not alter the surface tension lowering properties of the fluid, as shown by Gardner et al. (1971) and Huber et al. (1971) when using high levels of O_3 ($\geq$ 9800 μg/m^3; 5 ppm).

9.3.3.8 Lung Permeability. Table 9-10 summarizes studies of the effects on lung permeability of exposures to different concentrations of O_3.

The lung possesses several active-transport mechanisms for removal of substances from the airways to the capillary circulation. These removal mechanisms have been demonstrated to be carrier-mediated and specific for certain ions. Williams et al. (1980) studied the effect of O_3 on active transport of phenol red in the lungs of rats exposed to 1176 to 4116 μg/m^3 (0.6 to 2.1 ppm) of O_3 continuously for 24 hr. Ozone inhibited the carrier-mediated transport of intratracheally instilled phenol red from the lung to the circulation and increased the nonspecific diffusion of phenol red from the lung. These changes in ion permeability may also explain in part the effects of O_3 on the respiratory response of animals to bronchoconstrictors (Lee et al., 1977; Abraham et al., 1980).

As another index of increased lung permeability following O_3 exposure, the appearance of albumin and immunoglobins in airway secretions has been examined. Reasor et al. (1979) found that dogs breathing 1960 to 2940 μg/m^3 (1.0 to 1.5 ppm) of O_3 had increased albumin and immunoglobin G content of their airway secretions. Alpert et al. (1971a), using rats exposed for 6 hr to 490 to 4900 μg/m^3 (0.25 to 2.5 ppm) O_3, also found increased albumin in lung lavage in animals exposed to 980 μg/m^3 (0.5 ppm) or more.

In a series of experiments, Hu et al. (1982) exposed guinea pigs to 196, 510, 1000, or 1960 μg/m^3 (0.1, 0.26, 0.51, or 1.0 ppm) of O_3 for 72 hr and found increased lavage fluid protein content sampled immediately after exposure to concentrations $\geq$ 510 μg/m^3 (0.26 ppm) as compared with controls. Ozone-exposed guinea pigs had no accumulation of proteins when the exposure time was

TABLE 9-10. EFFECTS OF OZONE ON LUNG PERMEABILITY

Ozone concentration µg/m³	ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
196 510 1000 1960	0.1 0.26 0.51 1.0	CHEM, UV	3 hr or 72 hr	Increased levels of lavage fluid protein ≥ 0.26 ppm immediately after 72-hr exposure or 15 hr after a 3-hr exposure. Vitamin C deficiency did not influence sensitivity.	Guinea pig	Hu et al., 1982
353	0.18		3 hr; 8 hr/day for 5 or 10 days	No effect on protein levels.		
510 980	0.26 0.5		3, 24, or 72 hr	Lavage was 15 hr postexposure. At 0.26 ppm increased protein only after 24 hr exposure; at 0.5 ppm, increased protein after 24 hr of exposure.		
1000	0.51		3 hr	Increase in protein levels at 10- and 15-(but not 0, 5, or 24) hr postexposure.		
490 980 1960 4900	0.25 0.5 1.0 2.5	MAST, NBKI	6 hr	Increased alveolar protein accumulation at 0.5 ppm and above.	Rat	Alpert et al., 1971a
1176 2156 3136 4116	0.6 1.1 1.6 2.1	NBKI	24 hr	Concentration-dependent loss of carrier-mediated transport for phenol red.	Rat	Williams et al., 1980
1960- 2940	1.0- 1.5	MAST	2 hr	Increased albumin and immunoglobin G in airway secretions.	Dog	Reasor et al., 1979

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence; UV = UV photometry; NBKI = neutral buffered potassium iodide.

[b] Calibration method: NBKI = neutral buffered potassium iodide; UV = UV photometry.

reduced from 72 to 3 hr, unless the time of lavage was delayed for 10 to 15 hr following exposure. The protein content of the lavage fluid determined 10 to 15 hr following a 3-hr exposure increased in a concentration-related manner from 500 to 1470 $\mu g/m^3$ (0.256 to 0.75 ppm). Again 196 $\mu g/m^3$ (0.1 ppm) had no effects. The lavage fluid protein content of guinea pigs exposed to 353 $\mu g/m^3$ (0.18 ppm) of O_3 for 8 hr per day for 5 or 10 consecutive days was not different from air controls. No effect of vitamin C deficiency could be found on the accumulation of the lavage fluid protein in guinea pigs exposed to 196 to 1470 $\mu g/m^3$ (0.10 to 0.75 ppm) O_3 for 3 hr (Hu et al., 1982). In contrast, vitamin C-deficient guinea pigs have increased sensitivity to NO_2 (Selgrade et al., 1981). Polyacrylamide gel electrophoresis of lavage fluid proteins from animals exposed for 3 hr to 196 to 1470 $\mu g/m^3$ (0.1 to 0.75 ppm) O_3 showed the appearance of extra protein bands which co-migrated with serum proteins and of increased intensity of bands that also occur in air-exposed controls. This led the authors to conclude that the main source of the increased protein was serum.

Prostaglandins are intermediates in tissue edema resulting from a wide variety of mechanisms of injury. Increased lung permeability and edema produced by O_3 might also be mediated by prostaglandins. Non-steroidal anti-inflammatory drugs (aspirin and indomethacin) at appropriate doses inhibit lung edema in rats from exposure to 7890 $\mu g/m^3$ (4 ppm) of O_3 for 4 hr (Giri et al., 1975). Prostaglandins $F_{2\alpha}$ and E_2 were markedly increased in plasma and lung lavage of rats exposed to 7840 $\mu g/m^3$ (4 ppm) for up to 8 hr (Giri et al., 1980). Ozonolysis of arachidonic acid _in vitro_ produces fatty acid peroxides and other products having prostaglandin-like activity (Roycroft et al., 1977). Fatty acid cycloperoxides are produced directly by ozone-catalyzed peroxidation (Pryor, 1976; Pryor et al., 1976). Acute exposure to 5880 $\mu g/m^3$ (3 ppm) O_3 inhibits uncompetitively rat lung prostaglandin cyclooxygenase (Menzel et al., 1976).

Earlier reports that prostaglandin synthesis inhibitors exacerbated O_3-produced edema (Dixon and Mountain, 1965; Matzen, 1957a,b), as did methyl prednisolone (Alpert et al., 1971a), tend to confuse the interpretation of the role of prostaglandin in O_3-produced injury. Prostaglandin synthesis inhibitors clearly inhibit the degradation of prostaglandins and alter the balance between alternative pathways of fatty acid peroxide metabolism. Thus, while prostanoids are highly likely to be involved in O_3-produced edema, their exact role is still unexplained.

9.3.3.9 Proposed Molecular Mechanisms of Effects. Experts generally agree that the toxicity of O_3 depends on its oxidative properties. The precise mechanism of O_3's toxicity at the subcellular level is unclear, but several theories have been advanced. These theories include the following:

1. Oxidation of polyunsaturated lipids contained mainly in cell membranes;
2. Oxidation of sulfhydryl, alcohol, aldehyde, or amine groups in low molecular weight compounds or proteins;
3. Formation of toxic compounds (ozonides and peroxides) through reaction with polyunsaturated lipids;
4. Formation of free radicals, either directly or indirectly, through lipid peroxidation; and
5. Injury mediated by some pharmacologic action, such as via a neurohormonal mechanism, or release of histamine.

These mechanisms have been discussed in several reviews: U.S. Environmental Protection Agency (1978); National Air Pollution Control Administration (1970); North Atlantic Treaty Organization (1974); National Research Council (1977); Shakman (1974); Menzel (1970, 1976); Nasr (1967); Cross et al. (1976); Pryor et al. (1983); and Mudd and Freeman (1977). From these reviews and recent research detailed here or in previous biochemistry sections, two hypotheses are favored and may in fact be related.

9.3.3.9.1 Oxidation of polyunsaturated lipids. The first hypothesis is that O_3 initiates peroxidation of polyunsaturated fatty acids (PUFA) to peroxides, which produces toxicity through changes in the properties of cell membranes. Ozone addition to ethylene groups of PUFA can take place in membranes yet give rise to water-soluble products that can find their way to the cytosol. Aldehydes, peroxides, and hydroxyl radicals formed by peroxidation all can react with proteins. In addition to the direct oxidation of amino acids by O_3, secondary reaction products from O_3-initiated PUFA peroxidation can also oxidize amino acids or react with proteins to alter the function of the proteins. Since large numbers of proteins are embedded with lipids in membranes and rely on the associated lipids to maintain the tertiary structure of the protein, alterations in the lipid surrounding the protein can result in structural changes of the membrane-embedded protein. At present, methods are not available to differentiate effects on membrane proteins from effects on membrane lipids.

Some of the strongest evidence that the toxic reaction of O_3 can be associated with the PUFA of membranes is the protective effect of dietary vitamin E on O_3 toxicity (Roehm et al., 1971a, 1972; Chow and Tappel, 1972; Fletcher and Tappel, 1973; Donovan et al., 1977; Sato et al., 1976a; Chow and Kaneko, 1979; Plopper et al., 1979; Chow et al., 1981; Chow, 1983; Mustafa et al., 1983; Mustafa, 1975). Generally, vitamin E reduced the O_3-induced increase in enzyme activities of the glutathione peroxidase system (Section 9.3.3.2) and those involved in oxygen consumption (Section 9.3.3.3). More details are provided in Table 9-5. Morphological effects due to O_3 exposure are also lessened by dietary vitamin E (Section 9.3.1.4.1.1). Although this is not the strongest evidence, vitamin E supplementation also prevented O_3-induced changes in red blood cells (Chow and Kaneko, 1979).

These data consistently show that vitamin E has a profound effect on the toxicity of O_3 in animals. They also support indirectly lipid peroxidation as a toxic lesion in animals. However, although the influence of dietary vitamin E is clear, its relation to vitamin E levels in the lung, where presumably most lipid peroxidation would occur, is poorly understood. Rats of various ages (5, 10 and 90 days old and 2 yrs old) were fed normal diets; and 90-day-old rats were fed diets containing 0, 200, and 3000 mg of vitamin E/kg diet (Stephens et al., 1983). (Most of the biochemical studies of vitamin E protective effects were conducted with diets having far less than 200 mg/kg diet.) Rats were exposed to 1764 $\mu g/m^3$ (0.9 ppm) O_3 continuously for 72 hr, and periodic morphological observations were made. Those animals on normal diets had equivalent levels of vitamin E in the lung, but responses of the different age groups differed. Animals of a given age (90 days) maintained on the three vitamin E diets had different levels of vitamin E in the lungs (5.3 to 325 μg of vitamin E/g of tissue), but morphological responses were very similar. Stephens et al. concluded that O_3-induced responses in the lung are independent of the vitamin E content of the lung. Independent interpretation of this study is not possible, since very minimal descriptions of responses were provided and the number of animals was not given. Also, others (Chow et al., 1981; Plopper et al., 1979) found that for a given age of rat, different dietary levels of vitamin E (and perhaps different lung levels as shown by Stephens et al., 1983) influenced the morphological responses of rats to O_3.

9.3.3.9.2 Oxidation of sulfhydryl or amine groups. The second hypothesis is that ozone exerts its toxicity by the oxidation of low-molecular-weight compounds containing thiol, amine, aldehyde, and alcohol functional groups and by oxidation of proteins. Mudd and Freeman (1977) present a summary of the arguments for oxidation of thiols, amines, and proteins as the primary mechanism of O_3 toxicity based upon *in vitro* exposure data. Amino acids are readily oxidized by O_3 (Mudd et al., 1969; Mudd and Freeman, 1977; Previero et al., 1964). In the following descending order of rate, O_3 oxidizes the amino acids cysteine > methionine $\geq$ tryptophan > tyrosine > histidine > cystine > phenylalanine. The remaining common amino acids are not oxidized by O_3. Thiols are the most readily oxidized functional groups of proteins and peptides (Mudd et al., 1969; Menzel, 1971). Tryptophan in proteins is also oxidized *in vitro* by O_3 as shown by studies of avidin, the biotin-binding protein. Oxidation of tyrosine in egg albumin by O_3 occurs *in vitro*, converting the O_3-oxidized egg albumin to a form immunologically distinct from native egg albumin (Scheel et al., 1959). Ozone inactivated human alpha-1-protease inhibitor *in vitro* (Johnson, 1980). When treated with O_3, alpha-1-protease inhibitor lost its ability to inhibit trypsin, chymotrypsin, and elastase.

Meiners et al. (1977) found that O_3 reacted *in vitro* with tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, and 5-hydroxyindolacetic acid. One mole of O_3 was rapidly consumed by each mole of indole compound. Oxidation of tryptophan by O_3 also generates hydrogen peroxide. Hydrogen peroxide is a toxic substance in itself and initiates peroxidation of lipids (McCord and Fridovich, 1978). Other active O_2 species such as HO· and O_2^- are formed from hydrogen peroxide (McCord and Fridovich, 1978).

The results of the oxidation of functional groups in proteins can be generally observed by reduction of enzyme activities at high concentrations of O_3 (viz., 1960 to 7840 $\mu g/m^3$, 1 to 4 ppm for several hours). Many enzymes examined (Tables 10-5 to 10-10) in tissues have decreased activities (in many cases not statistically significant) immediately following even lower level (1960 $\mu g/m^3$, 1 ppm) O_3 exposures. The enzymes decreased include those catalyzing key steps supplying reduced cofactors to other processes in the cell, such as glucose-6-phosphate dehydrogenase (DeLucia et al., 1972) and succinate dehydrogenase (Mountain, 1963). Cytochrome P-450 (Goldstein et al., 1975), the lecithin synthetase system (Kyei-Aboagye et al., 1973), lysozyme (Holzman et al., 1968), and the prostaglandin synthetase system (Menzel et al., 1976) are decreased by O_3 exposure. Respiratory control of mitochondria is lost, and mitochondrial energy production is similarly decreased (Mustafa and Cross, 1974).

Mudd and Freeman (1977) point out that proteins are the major component of nearly all cell membranes, forming 50 to 70 percent by weight. The remainder of the weight of the cell membranes is lipids (phospholipids, glycolipids, glycerides, and cholesterol). Polyunsaturated fatty acids are components of membrane phospholipids, glycolipids, and glycerides. Mudd and Freeman contend, however, that proteins are far more easily oxidized by O_3 than are lipids. Indirect evidence in support of the idea that amines in particular are oxidized preferentially by O_3 is the protective effect of p-aminobenzoic acid (Goldstein et al., 1972a). Rats injected with p-aminobenzoic acid were partially protected from the mortality due to high concentrations of O_3. Presumably, the added p-aminobenzoic acid is oxidized by O_3 in place of proteins. Goldstein and Balchum (1974) later suggested that the protection of p-aminobenzoic acid, allylisopropylacetamide, and chlorpromazine was due to the induction of mixed function oxidase systems rather than a direct free radical scavenging effect. However, they also recognized that chlorpromazine can mask free radicals and result in membrane stabilization, which could account for the protective effects of these compounds preventing edema and inflammation. Acetylcholinesterase found on red blood cells is protected from inhibition by *in vitro* exposure to 78,400 $\mu g/m^3$ (40 ppm) O_3 through p-aminobenzoic acid treatment (Goldstein et al., 1972a). Amines are also efficient lipid antioxidants, so the results of these experiments could be interpreted in favor of the theory of peroxidation of the cell membrane as a mechanism of toxicity, as well.

9.3.3.9.3 Formation of toxic compounds through reaction with polyunsaturated lipids. The effects of O_3 could be due to the elaboration of products of peroxidation as well as peroxidation of the membrane itself. Menzel et al. (1973) injected 10 pg to 10 μg of fatty acid ozonides from oleic, linoleic, linolenic, and arachidonic acids into animals and found increased vascular permeability measured by extravascularly located Pontamine Blue dye bound to serum proteins. Extravascularization of serum proteins could be blocked by simultaneous antihistamine injection or by prior treatment with compound 48/80, a substance that depletes histamine stores. Cortesi and Privett (1972) injected methyl linoleate ozonide into rats or gave the compound orally; in each case acute pulmonary edema resulted. The pulmonary edema and changes in fatty acid composition of the serum and lung lipids were similar to those occurring after O_3 exposure (Roehm et al., 1971a,b, 1972; Menzel et al., 1976).

Peroxidation of lung lipids could lead to cytotoxic products. Phosphatidyl choline liposomes (spheres formed by emulsifying phosphatidyl choline in water) were lysed on exposure to O_3 (Teige et al., 1974). Liposomes exposed to O_3 were more active than O_3 alone in lysing red blood cells. The products of ozonolysis of phosphatidyl choline could be stable, yet toxic, intermediates.

9.3.3.9.4 Formation of free radicals and injury mediated by pharmacologic action. The other theories may be linked to the consequences of peroxidation of PUFA. Ozonation of PUFA results in the formation of peroxides (e.g., ROOH or ROOR) rather than oxidation of alkenes to higher oxidation states (e.g., ROH, RCHO or RCO2H). Peroxides (ROOH or ROOR) are chemically reactive and may be the ultimate toxicants, not simply products of oxidation. During the process of peroxidation or direct addition of O_3 to PUFA, free radicals may be generated (Pryor et al., 1983), and these free radicals may be the ultimate toxicants.

Because the metabolism of PUFA peroxides in the lungs is intimately linked with the metabolism of thiol compounds (such as glutathione, GSH), direct oxidation of thiols by O_3 (hypothesis B) may link the two major hypotheses A and B with hypothesis C, formation of toxic products. Ozone depletion of GSH could render the peroxide detoxification mechanism ineffective. However, at levels of O_3 below 1960 $\mu g/m^3$ (1 ppm), increases in glutathione have been observed (Plopper et al., 1979; Fukase et al., 1975; Moore et al., 1980; Mustafa et al., 1982). The rat lung is sensitive to the increase of glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase activities at levels as low as 196 $\mu g/m^3$ (0.1 ppm) O_3 continuously for 7 days (Plopper et al., 1979; Chow et al., 1981; Mustafa, 1975; Mustafa and Lee, 1976) in vitamin E-deficient rats. With vitamin E-supplemented rats, 392 $\mu g/m^3$ (0.2 ppm) caused similar effects. After acute exposures to >1960 $\mu g/m^3$ (1 ppm) O_3, decreases are observed in these enzyme activities and glutathione levels. Other species (mice and monkeys) exhibit similar effects at different concentrations (Table 9-5). These changes at the lower levels of O_3 appear to be coincidental with the initiation of repair and proliferative phases of lung injury (Cross et al., 1976; Dungworth et al., 1975a,b; Mustafa and Lee, 1976; Mustafa et al., 1977, 1980; Mustafa and Tierney, 1978). The parallel increase in the number of type 2 cells having higher levels of metabolic activity would be expected to cause an overall increase in the metabolic activity of the lung. This was substantiated by Mustafa (1975) and DeLucia et al. (1975a), who found that the O_2 consumption per mitochondrion was not increased in O_3-exposed lungs but that the number of mitochondria was increased. Chow and Tappel (1972) proposed that alterations of enzymatic activity were due to stimulation of glutathione peroxidase pathway. Chow and Tappel (1973) also proposed that the changes in the pentose shunt and glycolytic enzymes in lungs of the O_3-exposed rats were due to the demands of the glutathione peroxidase system for reducing equivalents in the form of NADPH. Cross et al. (1976) and DeLucia (1975a,b) reported that O_3 exposure oxidized glutathione and formed mixed disulfides between proteins and non-protein sulfhydryl compounds.

The general importance of glutathione in preventing lipid peroxidation in the absence of O_3 has been shown by Younes and Siegers (1980) in rat and mouse liver where depletion of glutathione by treatment with vinylidene chloride or diethylmaleate led to increased spontaneous peroxidation. These authors suggest that glutathione prevents spontaneous peroxidation by suppression of radicals formed by the enzyme cytochrome P-450 or already produced hydroperoxides.

Chow and Tappel (1973) suggest that peroxides formed via lipid peroxidation increase glutathione peroxidase activity and, in turn, increase levels of the enzymes necessary to supply reducing equivalents (NADPH) to glutathione reductase. Vitamin E suppresses spontaneous formation of lipid peroxides and, therefore decreased the glutathione peroxidase activity in mouse red cells (Donovan and Menzel, 1975; Menzel et al., 1978). The supplementation of rats with vitamin E could, therefore, decrease the utilization of glutathione by spontaneous reaction or by ozone-initiated peroxidation. The two mechanisms could then interact in a concerted fashion to decrease ozone cytotoxicity. Eliminating vitamin E from the diet increases the chances of increases of this system.

In support of hypothesis E, Wong and Hochstein (1981) found that thyroxin enhanced the osmotic fragility of human erythrocytes exposed to O_3 in vitro. They found also that ^{125}I from radiolabeled thyroxin was incorporated into the major membrane glycoprotein, glycophorin, of red cells. When these events had occurred, the cation permeability of the human red cells was enhanced without measurable inhibition of ATPase or membrane lipid peroxidation. They suggested that thyroid hormones play an important role in O_3 toxicity. Fairchild and Graham (1963) found that thyroidectomy, thiourea, and antithyroid drugs protected animals from lethal exposures to O_3 and nitrogen dioxide. Fairchild and

Graham ascribed the mortality following O_3 to pulmonary edema. Wong and Hochstein (1981) suggested that O_3 toxicity in the lung may be altered through a free radical mechanism involving iodine transfer from thyroxin to lung membranes. The hormonal status of animals could alter a variety of defense mechanisms and O_3 sensitivity.

9.3.3.9.5 Summary. The actual toxic mechanism of O_3 may involve a mixture of all of these chemical mechanisms because of the interrelationships between the peroxide detoxification mechanisms and glutathione (See Section 9.3.3.2) and the complexity of the products produced from ozonation of PUFA. A single chemical reaction may not be adequate to explain O_3 toxicity. The relative importance of any one reaction, oxidation of proteins, PUFA, or small molecular weight compounds, will depend upon a number of factors such as the presence of enzymatic pathways of decomposition of products formed (peroxides), pathways for regeneration of thiols, the presence of non-enzymatic means of terminating free radical reactions (vitamins E and C), and differences in membrane composition of PUFA (relative ease of attack of O_3), for example.

9.3.4 Effects on Host Defense Mechanisms

The mammalian respiratory tract has a number of closely coordinated pulmonary defense mechanisms that, when functioning normally, provide protection from the adverse effects of a wide variety of inhaled microbes and other particles. A variety of sensitive and reliable methods have been used to assess the effects of O_3 on the various components of this defense system to provide a better understanding of the health effects of this pollutant.

The previous Air Quality Criteria Document for Ozone and Photochemical Oxidants (U.S. Environmental Protection Agency, 1978) provided a review and evaluation of the scientific literature published up to 1978 regarding the effects of O_3 on host defenses. Other reviews have recently been written that provide valuable references to the complexity of the host defense system and the effects of environmental chemicals such as O_3 on its integrity (Gardner, 1981; Ehrlich, 1980; Gardner and Ehrlich, 1983; Goldstein, 1984).

This section describes the existing data base and, where appropriate, provides an interpretation of the data, including an assessment of the different microbial defense parameters used, their sensitivity in detecting abnormalities, and the importance of the abnormalities with regard to the pathogenesis of infectious disease in the exposed host. This section also discusses the various components of host defenses, such as the mucociliary escalator and the alveolar macrophages, which clear the lung of both viable and nonviable particles, and integrated mechanisms, which are studied by investigating the host's response to experimentally induced pulmonary infections. The immune system, which defends the overall host against both infectious and neoplastic diseases, is also discussed.

9.3.4.1 Mucociliary Clearance. The mucociliary transport system is one of the lung's primary defense mechanisms against inhaled particles. It protects the conducting airways by trapping and quickly removing material that has been deposited on the mucociliary escalator. The effectiveness of mucociliary clearance can be determined by measuring such biological activities as the rate of transport of deposited particles; the frequency of ciliary beating; structural integrity of the ciliated cells; and the size, number, and distribution of mucus-secreting cells. Once this defense mechanism has been altered, a buildup of both nonviable and possibly viable inhaled substances can occur on the epithelium and may jeopardize the health of the host, depending on the nature of the uncleared substance. As an example, regardless of the severity of the influenza infection, the virus concentrates initially in the lining of the airways. The virus then spreads to alveolar cells of the lung parenchyma. It is suspected that the macrophage might be the site of replication of this virus (Yilyma et al., 1979; Nayak et al., 1964; Raut et al., 1975).

A number of studies with various animal species have reported morphological damage to the cells of the tracheobronchial tree from acute and subchronic exposure to 490 to 1960 μg/m^3 (0.20 to 1.0 ppm) of O_3. (See Section 9.3.1.) The cilia were either completely absent or had become noticeably shorter or blunt. By removing these animals to a clean-air environment, the structurally damaged cilia regenerated and appeared normal. Based on such morphological observations, related effects such as ciliostasis, increased mucus secretions, and a slowing of mucociliary transport rates might be expected. However, no measurable changes in ciliary beating activity have been reported due to O_3 exposure alone. Assay of isolated tracheal rings from hamsters immediately after a 3-hr exposure to 196 μg/m^3 (0.1 ppm) of O_3 showed no significant loss in ciliary beating activity (Grose et al., 1980). In the same study, when the animals were subsequently exposed for 2 hr to 1090 μg/m^3 H_2SO_4 (0.30 μm volume median diameter), a significant reduction in ciliary beating frequency occurred. The magnitude of this effect was, however, significantly less than that observed due to the effect of H_2SO_4 exposure alone. In either case, the animals completely recovered within 72 hr when allowed to remain in a clean-air environment. These authors found only a slight decrease in beating frequency with a simultaneous exposure to 196 $\mu g/m^3$ (0.1 ppm) of O_3 and 847 $\mu g/m^3$ H_2SO_4 (Grose et al., 1982). These data indicate that O_3 appears to partially protect against the effects of H_2SO_4 on ciliary beating frequency. Grose et al. (1982) proposed that the ciliary cells may be partially protected due to the increase in mucus tracheal secretion of glycoproteins (Last and Cross, 1978) resulting from exposure to these chemicals.

Studies cited in the previous criteria document (U.S. Environmental Protection Agency, 1978) gave evidence on the effect of O_3 on the host's ability to physically remove deposited particles (Table 9-11). The slowing of mucus transport in both rat and rabbit trachea as a result of O_3 exposure was reported in the early literature (Tremer et al., 1959; Kensler and Battista, 1966). Goldstein, E., et al. (1971a,b, 1974) provided evidence that the primary effect of O_3 on the defense mechanism of the mouse lung was to diminish bactericidal activity but not to significantly affect physical removal of the deposited bacteria. In these studies, mice were exposed to an aerosol of ^{32}P-labeled Staphylococcus aureus either after a 17-hr exposure to O_3 or before a 4-hr exposure. Concentrations of O_3 were 1180, 1370, 1570, or 1960 $\mu g/m^3$ (0.6, 0.7, 0.8, or 1.0 ppm). The physical clearance and bactericidal capabilities of the lung were then measured 4 to 5 hr after bacterial exposure. Exposure 17 hr before infection caused a significant reduction in bactericidal activity beginning at 1960 $\mu g/m^3$ (1.0 ppm) of O_3. When mice were exposed to O_3 for 4 hr after being infected, there was a significant decrease in bactericidal activity for each O_3 concentration, and with increasing O_3 concentration, there was a progressive decrease in bactericidal activity. The investigators proposed that because mucociliary clearance was unaffected by subsequent O_3 exposure, the bactericidal effect was due to dysfunction of the alveolar macrophage. Warshauer et al. (1974) reported that a deficiency in vitamin E would further reduce the lungs' bactericidal activity.

Friberg et al. (1972) studied the effect of a 16-hr/day exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3 on the lung clearance rate of radiolabeled monodisperse polystyrene and iron particles in the rabbit and of bacteria in the guinea

TABLE 9-11. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: DEPOSITION AND CLEARANCE

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effects	Species	Reference
μg/m³	ppm					
196	0.1	CHEM	3 hr	No effect on ciliary beating frequency.	Hamster	Grose et al., 1980
784	0.4	NBKI	3 hr	Initially lowers deposition of inhaled bacteria, but subsequently a higher number are present due to reproduction.	Mouse	Coffin and Gardner, 1972b
784	0.4	ND	4 hr	Bactericidal activity inhibited. Silicosis did not enhance O_3 effect.	Mouse	Goldstein et al., 1972b
785, 1568, 1960	0.4, 0.8, 1.0	UV	4 hr	Delay in mucociliary clearance, acceleration in alveolar clearance.	Rat	Kenoyer et al., 1981
1764	0.5	MAST	1.4 hr	Increases nasal deposition and growth of virus; no effect in the lungs.	Mouse	Fairchild, 1974, 1977
980	0.5	NBKI	16 hr/day, 7 months	No effect on clearance of polystyrene and iron particles.	Rabbit	Friberg et al., 1972
980	0.5	NBKI	2 months	Reduced clearance of viable bacteria.	Guinea pig	Friberg et al., 1972
980, 1960	0.5, 1.0	CHEM	2 hr	Reduced tracheal mucus velocity at 1.0 ppm. No effect at 0.5 ppm.	Sheep	Abraham et al., 1980
784-3979	0.57-2.03	M	17 hr before bacteria	Physical clearance not affected, but bactericidal activity affected at 0.99 ppm. Decrease in deposition of inhaled organisms at 0.57 ppm.	Mouse	Goldstein et al., 1971a

TABLE 9-11. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: DEPOSITION AND CLEARANCE (continued)

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a] method	Exposure duration and protocol	Observed Effects	Species	Reference
1176	0.62-4.25	M	4 hr after bacteria	No effect on bacterial deposition and clearance; reduced bactericidal activity at each exposure level.	Mouse	Goldstein et al., 1971b
1372	0.7	M	7 days	Deficiency of Vitamin E further reduced bactericidal activity after 7 days.	Rat	Warshauer et al., 1974
1372	0.7	G	3-4 hr	Reduced bactericidal activity in lungs.	Mouse	Bergers et al., 1982
1568	0.8	UV	4 hr	Slowed tracheobronchial clearance and accelerated alveolar clearance. Effects greater with higher humidity.	Rat	Phalen et al., 1980
1960	1.0	NBKI	3 hr	Bacteria clear lung and invade blood.	Mouse	Coffin and Gardner, 1972b
2352	1.2	UV	4 hr	Delayed mucociliary clearance of particles.	Rat	Frager et al., 1979

[a] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; UV = UV photometry; NBKI = neutral buffered potassium iodide; MAST = KI-coulometric (Mast meter); M = microcoulomb sensor; G = galvanic meter.

pig. The results from the guinea pig studies showed a reduced clearance of viable bacteria. The rabbit's lung clearance rate was not affected by O_3. In this latter study, however, a large number of the test animals died during exposure from a respiratory disease, and the results must be viewed with caution.

Recent studies have continued to examine the effects of O_3 on mucociliary transport in the intact animal. Phalen et al. (1980) attempted to quantitate the removal rates of deposited material in the upper and lower respiratory tract of the rat. In this study, the clearance rates of radiolabeled monodisperse polystyrene latex spheres were followed after O_3 exposure. A 4-hr exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 significantly slowed the early (tracheobronchial) clearance and accelerated the late (alveolar) clearance rates at both low (30 to 40 percent) and high (> 80 percent) relative humidity. These effects were even greater at higher humidity, which produced nearly additive effects. Combining O_3 with various sulfates [$Fe_2(SO_4)_2$, H_2SO_4, $(NH_4)_2SO_4$] gave clearance rates very similar to those for O_3 alone. Accelerating the long-term clearance from the alveoli may not in itself be harmful; however, because this process may result from an influx of macrophages into the alveolar region, the accumulation of excess numbers of macrophages might present a potential health hazard because of their high content of proteolytic enzymes and O_2 free radicals, which have the capability for tissue destruction. Essentially no data are available on the effects of prolonged exposure to O_3 on ciliary functional activity or on mucociliary transport rates measured in the intact animal.

Frager et al. (1979) deposited insoluble, radioactive-labeled particles via inhalation and monitored the clearance rate after a 4-hr exposure to 2352 $\mu g/m^3$ (1.2 ppm) of O_3. This exposure caused a substantial delay in rapid (mucociliary) clearance in the rat. However, if the animals were exposed 3 days earlier to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for 4 hr, the pre-exposure eliminated this effect, resulting in a clearance rate that was essentially the same as for controls. Thus, the pre-exposure to a lower level 3 days before rechallenging with a higher concentration of O_3 appeared to afford complete protection at 3 days. After a 13-day interval between the pre-exposure and the challenges, this adaptation or tolerance was lost.

These results were confirmed when Kenoyer et al. (1981) repeated these studies, with three different concentrations of O_3, 784, 1568, and 1960 $\mu g/m^3$ (0.4, 0.8, and 1.0 ppm). At each of the three concentrations, a delay was observed in early (0 to 50 hr postdeposition of particles) clearance, and an acceleration was seen in long-term (50 to 300 hr postdeposition) clearance, as compared to controls. Concentration-response curves showed that clearance was affected more by the higher concentrations of O_3.

The velocity of the tracheal mucus of sheep was not significantly altered from a baseline value of 14.1 mm/min after a 2-hr exposure to 980 $\mu g/m^3$ (0.5 ppm) of O_3 (Abraham et al., 1980). The authors state that 1960 $\mu g/m^3$ (1 ppm) of O_3 for 2 hr did significantly reduce, both immediately and 2 hr postexposure, the tracheal mucus velocity.

9.3.4.2 Alveolar Macrophages. Within the gaseous exchange region of the lung, the first line of defense against microorganisms and nonviable insoluble particles is the resident population of alveolar macrophages. These cells are responsible for a variety of important activities, including detoxification and removal of inhaled particles, maintenance of pulmonary sterility, and interaction with lymphoid cells for immunological protection. In addition, macrophages act as scavengers by removing cellular debris. To adequately fulfill their purpose, these defense cells must maintain active mobility, a high degree of phagocytic activity, an integrated membrane structure, and a well-developed and functioning enzyme system. Table 9-12 illustrates the effects of O_3 on the alveolar macrophage (AM).

Under normal conditions, the number of free AMs located in the alveoli is relatively constant when measured by lavage (Brain et al., 1977, 1978). Initially, O_3, through its cytolytic action that is probably mediated through its action on the cell membrane, significantly reduces the total number of these defense cells immediately after exposure (Coffin and Gardner, 1972b). The host responds with an immediate influx of cells to aid the lung in combating this assault. Little is known about the mechanisms of action that stimulate this migration or about the fate of these immigrant cells. The source of these new cells may be either (1) the influx of interstitial macrophages, (2) the proliferation of interstitial macrophagic precursors with subsequent migration of the progeny into the air space, (3) migration of blood monocytes, or (4) division of free AMs. The rapid increase in the number of macrophages is evidently a biphasic response, arising from an early phase

TABLE 9-12. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: MACROPHAGE ALTERATIONS

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effects[c]	Species	Reference
μg/m³	ppm					
196 1960	0.1 1.0	NBKI	2.5 hr or 30 min *in vitro*	Lung protective factor partially inactivated, increasing fragility of macrophages (concentration-related).	Rabbit	Gardner et al., 1971
392	0.2	MAST NBKI	8 hr/day for 7 days	Increased number of macrophages in lungs (morphology).	Monkey Rat	Castleman et al., 1977 Dungworth, 1976 Stephens et al., 1976
490 980	0.25 0.50	NBKI	3 hr (*in vivo* and *in vitro*)	Decreased activity of the lysosomal enzymes lysozyme, acid phosphatase, and β-glucuronidase.	Rabbit	Hurst et al., 1970 Hurst and Coffin, 1971
980	0.5	NBKI	8 hr/day for 7 days	Increased osmotic fragility.	Rabbit	Dowell et al., 1970
980	0.5	NBKI	3 hr	Decreased enzyme activity and increased influx of PMNs.	Rabbit	Alpert et al., 1971b
980	0.5	CHEM	3 hr	Decreased red blood cell rosette binding to macrophages.	Rabbit	Hadley et al., 1977
980 1313	0.5 0.67	NBKI	3 hr	Decreased ability to ingest bacteria.	Rabbit	Coffin et al., 1968 Coffin and Gardner, 1972b
980 1960	0.5 1	NBKI	2 hr (*in vitro*)	Decreased agglutination in the presence of concanavalin A.	Rat	Goldstein et al., 1977
1058	0.54	UV	23 hr/day for 34 days	Increased number of macrophages (morphological).	Mouse	Zitnik et al., 1978

TABLE 9-12. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: MACROPHAGE ALTERATIONS (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effects[c]	Species	Reference
μg/m³	ppm					
1568	0.8	ND	11 days	No effect on in vitro interferon production with alveolar macrophages but did inhibit the production of interferon by tracheal epithelial cells.	Mouse	Ibrahim et al., 1976
1568	0.8	ND	90 days	Eightfold increase in number of macrophages at 7 days, reducing to fourfold after 90 days.	Rat	Boorman et al., 1977
1568	0.8	UV	7 days	Decreased number of migrating macrophages and total distance migrated.	Monkey	Schwartz and Christman, 1979
1568	0.8	MAST	3, 7, 20 days	Increased phagocytosis.	Rat	Christman and Schwartz, 1982
1960 9800	1 5	CHEM	3 hr	Decreased ability to produce interferon in vitro.	Rabbit	Shingu et al., 1980
1960	1	UV	4 hr	Decreased in vitro migrational ability, as evidenced by decreased number of macrophages able to migrate.	Rat	McAllen et al., 1981
3136 6860	1.6 to 3.5	NBKI	2 hr to 3 hr	Decreased superoxide anion radical production.	Rat	Amoruso et al., 1981 Witz et al., 1983
4900	2.5	M	5 hr	Loss of β-glucuronidase and acid phosphatase in PAM with ingested bacteria; decreased rate of bacterial ingestion.	Rat	Goldstein et al., 1978b
4900	2.5	M	5 hr	Diminished rate of bacterial killing, increased numbers of intracellular staphylococcal clumps; lack of lysozyme in macrophages with staphylococcal clumps.	Rat	Kimura and Goldstein, 1981

[a] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; UV = UV photometry; NBKI = neutral buffered potassium iodide; MAST = KI-coulometric (Mast meter); M = microcoulomb sensor.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] Abbreviations used: PMN = polymorphonuclear leukocytes; PAM = pulmonary alveolar macrophage.

apparently correlated to a local cellular response and a later phase of interstitial cell proliferation, which is responsible for the maintenance of the high influx of macrophages (Brain et al., 1978).

Morphological studies have supported the observation that exposure to O_3 can result in a macrophage influx in several animal species. Exposure of mice to 1058 $\mu g/m^3$ (0.54 ppm) of O_3 23 hr/day for a maximum of 34 days resulted in an increased number of macrophages within the proximal alveolar ducts (Zitnik et al., 1978). These cells were highly vacuolated and contained many secondary phagocytic vacuoles filled with cellular debris. The effect was most prominent after 7 days of exposure and became less evident as the exposure continued. This observation correlated with the finding in rats of an eightfold increase in the number of pulmonary free cells after exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for 7 days, but only a fourfold increase after exposure for 90 days (Brummer et al., 1977; Boorman et al., 1977). In similar studies, other authors found that exposure of both monkeys and rats to concentrations as low as 392 $\mu g/m^3$ (0.2 ppm) of O_3 for 8 hr/day on 7 consecutive days resulted in an accumulation of macrophages in the lungs of these exposed animals (Castleman et al., 1977; Dungworth, 1976; Stephens et al., 1976). The data from these studies suggest that these two species of animals are approximately equal in susceptibility to the short-term effects of O_3.

Thus, the total available data would indicate that, after short periods of O_3 insult, there is a significant reduction in the number of free macrophages available for pulmonary defense, and that these macrophages are more fragile, are less phagocytic, and have decreased enzymatic activity (Dowell et al., 1970; Coffin et al., 1968; Coffin and Gardner, 1972b; Hurst et al., 1970). However, histological studies have reported that with longer exposure periods, there is an influx and accumulation of macrophages within the airways. Such a marked accumulation of macrophages within alveoli may appear to be a reasonable response to the immediate insult, but it has been speculated that the consequences of this mass recruitment may also be instrumental in the development of future pulmonary disease due to the release of proteolytic enzymes by the AMs (Brain, 1980; Menzel et al., 1983).

A number of integrated steps are involved in phagocytosis processes, the first being the ability of the macrophage to migrate to the foreign substance on stimulus. McAllen et al. (1981) studied the effects of 1960 $\mu g/m^3$ (1.0 ppm) of O_3 for 4 hr on the migration rate of AMs. Migration was measured by determining the area macrophages could clear of gold-colloid particles that had been previously precipitated onto cover slips. In this study, it was not clear whether the gold was actually ingested or merely adhered to the outer surface of the cell. Nevertheless, the cells from O_3-exposed rats appeared less mobile, in that they migrated 50 percent less than the sham-exposed group.

A decrease in the ability of macrophages to phagocytize bacteria after exposure to concentrations as low as 980 $\mu g/m^3$ (0.5 ppm) of O_3 for 3 hr was demonstrated by Coffin et al. (1968) using rabbits. However, Christman and Schwartz (1982) may have demonstrated that with longer exposure periods, the effects may be different (i.e., the phagocytic rate may increase). In this study, rats were exposed to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for 3, 7 or 20 days; at those times the macrophages were isolated, allowed to adhere to glass, and incubated with carbon-coated latex microspheres. The percentages of phagocytic cells were determined at 0.25, 0.5, 1, 2, 4, 8 and 24 hr of incubation. At all exposure time periods tested, the number of spheres engulfed had increased. The greatest increase in phagocytic activity was observed after 3 days of exposure. The exposed cells engulfed a greater number of spheres than controls, and a larger percentage of macrophages from exposed animals was phagocytic. This enhancement correlated well with a significant increase in cell spreading of AMs from exposed rats as compared to controls. If longer-term O_3 exposure enhances macrophage function and causes a migration of macrophages into the lung, the comparisons of function of these new cells with controls may not be valid, because these new cells are biochemically younger. Another significant problem with this study is that the cells examined were a selected population because only the cells that adhered to the glass surface were available for study. The cells that did not adhere were removed by washing. In this study, only 51 percent of the collected cells adhered after 3 days of O_3 exposure, compared to 85 percent of the controls. Although the effects of O_3 on cell attachment have not been studied directly, there is evidence that O_3 affects AM membranes involved in the attachment process (Hadley et al., 1977; Dowell et al., 1970; Aranyi et al., 1976; Goldstein et al., 1977). The cells most affected by the cytotoxic action of the O_3 exposure might never have been tested, because they were discarded.

Goldstein et al. (1977) studied the effect of a 2-hr exposure on the ability of AM to be agglutinated by concanavalin-A, a parameter reflecting membrane organization. A decrease in agglutination of rat AMs was found after exposure to 980 or 1960 μg/m^3 (0.5 or 1.0 ppm) of O_3. A decrease in concanavalin-A agglutinability of trypsinized red blood cells obtained from rats exposed for 2 hr to 1960 μg/m^3 (1 ppm) was also noted. Hadley et al. (1977) investigated AM membrane receptors from rabbits exposed to 980 μg/m^3 (0.5 ppm) of O_3 for 3 hr. Following O_3 exposure, lectin-treated AMs have increased rosette formation with rabbit red blood cells. The authors hypothesized that the O_3-induced response indicates alterations of macrophage membrane receptors for the wheat germ agglutinin that may lead to changes in the recognitive ability of the cell.

Ehrlich et al. (1979) studied the effects of O_3 and NO_2 mixtures on the activity of AMs isolated from the lungs of mice exposed for 1, 2, and 3 months. Only after a 3-month exposure to the mixture of 196 μg/m^3 (0.1 ppm) of O_3 and 0.5 ppm of NO_2 (3 hr/day, 5 days/week) did viability in macrophages decrease significantly. In vitro phagocytic activity was also not affected by a 1-month exposure to this level of pollutants, but after 2- and 3-month exposures the percentage of macrophages that had phagocytic activity decreased significantly.

It has been reported that the acellular fluid that lines the lungs also plays an important role in defense of the lung through its interaction with pulmonary macrophages (Gardner et al., 1971; Gardner and Graham, 1977). These studies demonstrated that the protective components of this acellular fluid can be inactivated by a 2.5-hr exposure to O_3 levels as low as 196 μg/m^3 (0.1 ppm). When normal AM's are placed in fluid lavaged from O_3-exposed animals, they showed an increase in lysis (10 percent over control). A similar effect was seen when normal AM's were placed in protective fluid that had been exposed in vitro to O_3. The data indicate that some of the effects of O_3 on lung cells may be mediated through this lung lining fluid. Schwartz and Christman (1979) provided evidence that normal lung lining material enhanced macrophage migration, but the macrophages obtained from rhesus monkeys after exposure for 7 days to 1568 μg/m^3 (0.8 ppm) of O_3 demonstrated both a decrease in the number of cells that migrated (28 percent of control value) and in the total distance they traveled (71 percent of control value). Adding normal lining fluid to isolated O_3-exposed macrophages did enhance the migration, but it was still significantly less than controls.

Macrophages are rich in lysosomal enzymes. Because these enzymes are crucial in the functioning of the macrophage, perturbation of the metabolic or enzymatic mechanisms of these cells may have important consequences on the abilities of the lung to defend itself against disease. Enzymes that have been identified include acid phosphatase, acid ribonuclease, beta galactosidase, beta glucuronidase, cytochrome oxidase, lipase, lysozyme, and protease. Ozone decreased significantly the activity levels of lysozyme, β-glucuronidase, and acid phosphatase in macrophages after a 3-hr exposure of rabbits to concentrations as low as 490 $\mu g/m^3$ (0.25 ppm) of O_3 (Hurst et al., 1970). Such enzymatic reductions were also observed in AMs exposed *in vitro* (Hurst and Coffin, 1971). The ability of O_3 to alter macrophages' enzyme activity was also studied by means of unilateral lung exposure of rabbits (Alpert et al., 1971b). A significant reduction in these same three intracellular enzymes was found to be specific to the lung that breathed O_3 rather than a generalized systemic response. These effects were concentration-related, beginning at 980 $\mu g/m^3$ (0.5 ppm) of O_3. The extracellular release of such enzymes may occur either as a result of direct cytotoxic damage and leakage of intracellular contents, or they may be selectively released without any cell injury. Hurst and Coffin (1971) showed that the reduction in intracellular lysosomal enzyme activity observed after *in vitro* exposure coincides with the release of the enzyme into the surrounding medium. In these studies, the sum of the intra- plus extracellular enzyme activity did not equal the total activity, indicating that the pollutant itself can inactivate the hydrolytic enzyme as well as alter the cell membrane. Recently, Witz et al. (1983) and Amoruso et al. (1981) reported that *in vivo* O_3 exposure affected the production of superoxide anion radicals (O_2^-) by rat AMs. This oxygen radical is important in antibacterial activity. Exposure to concentrations above 3136 $\mu g/m^3$ (1.6 ppm) of O_3 for 2 hr appears to result in a progressive decrease in O_2^- production. No statistical evaluation of the data was performed. The type of membrane damage as well as the mechanisms by which this damage is incurred are not well understood. It is not known whether the O_3-induced inhibition of O_2^- production arises from the direct oxidative damage of the membrane enzyme involved in the metabolism of O_2 to O_2^-, or whether it is a result of oxidative degradation of membrane lipids that may serve a cofactor function.

Shingu et al. (1980) reported the effects of O_3 on the ability of two cell types, macrophages and tonsillar lymphocytes, to produce interferon, a substance that aids in defending the host organism against viral infections. Macrophages from rabbits exposed to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 for 3 hr exhibited a depression in interferon production. Interferon production by tonsillar lymphocytes was not significantly depressed by 9800 μg/m^3 (5.0 ppm) of O_3. The authors suggested that an impairment of interferon production might play an important role in the ability of the host to combat respiratory viral infections. In neither of the studies were statistical analyses of the data reported. Ibrahim et al. (1976) also exposed mice to O_3 and illustrated that 1568 μg/m^3 (0.8 ppm) of O_3 for a period of 11 days inhibited the *in vitro* ability of tracheal epithelial cells to produce interferon, but no effect was observed with alveolar macrophages.

9.3.4.3 *Interaction with Infectious Agents*. In general, the consequences of any toxic response depend on the particular cell or organ affected, the severity of the damage, and the capability of the impaired cells or tissue to recover from the assault. Do small decrements in the functioning of these various host defense mechanisms compromise the host so that it is unable to defend itself against a wide variety of opportunistic pathogens? It has been suggested from epidemiological data that exposure to ambient levels of oxidants can enhance the development of respiratory infection in humans (Dohan et al., 1962; Thompson et al., 1970). Measurement of the competency of the host's antimicrobial mechanisms can best be tested by challenging both the toxic-exposed animals and the clean-air exposed control animals to an aerosol of viable microorganisms. If the test substance, such as O_3, had any adverse influence on the efficiency of any of the host's many protective mechanisms (i.e., physical clearance via the mucociliary escalator, biocidal activity mediated through macrophages, and associated cellular and humoral immunological events) that would normally function in defense against a microbe, the microbe, in its attempt to survive, would take advantage of these weaknesses. A detailed description of the infectivity model commonly used for O_3 studies has been published elsewhere (Coffin and Gardner, 1972b; Ehrlich et al., 1979; Gardner, 1982a). Briefly, animals are randomly selected to be exposed to either clean air or O_3. After the exposure ends, the animals from both chambers are combined and exposed to an aerosol of viable microorganisms. The vast majority of these studies have been conducted with *Streptococcus sp.* At the termination of this 15- to 20-min exposure, the animals are housed in clean air, and the rate of mortality in the two groups is determined during a 15-day holding period. In this system, the concentrations of O_3 used do not cause any mortality. The mortality in the control group (clean air plus exposure to the microorganism) is approximately 10 to 20 percent and reflects the natural resistance of the host to the infectious agent. The difference in mortality between the O_3-exposure group and the controls is concentration-related (Gardner, 1982a). No studies have yet been conducted to determine the lowest possible number of viable microbes that when deposited in the lung will cause a pulmonary infection in the host exposed to O_3. Miller et al. (1978a) examined the cumulative data from nearly 3,000 control mice used in these infectivity studies and determined that within the range of 200 to 4,000 colony-forming units per lung, there is no correlation between number of bacteria deposited in the lung and the resulting increase in percent mortality. It must be noted that in these studies, the control animals are capable of maintaining pulmonary sterility by inactivating the microbes that are deposited in the lung.

If the test agent does not impair the host's defense mechanisms, there is a rapid inactivation of inhaled microorganisms that have been deposited in the respiratory system. However, if the chemical exposure alters the ability of these defense cells to function, i.e., rate of bacterial killing, the number of microbes in the lungs could increase (Coffin and Gardner, 1972b; Miller et al., 1978; Gardner, 1982a). This acceleration in bacterial growth has been attributed to the pollutant's alteration of the capability of the lung to destroy the inhaled bacteria, thus permitting those with pathogenic potential to multiply and produce respiratory pneumonia. With this accelerating growth, there is an invasion of the blood, and death has been predicted from a positive blood culture (Coffin and Gardner, 1972b).

Coffin et al. (1967) treated mice with O_3 (0.08 ppm and higher levels for 3 hr) and subsequently exposed them to an aerosol of infectious *Streptococcus sp.* In this study, O_3 increased the animals' susceptibility to infection, resulting in a significant increase in mortality rate in the O_3-treated group. Ehrlich et al. (1977), when using the same bacteria but a different strain of mice, found a similar effect at 0.1 ppm for 3 hr. When using CD-1 mice and *Streptococcus sp.*, Miller et al. (1978) studied the effects of a 3-hr exposure to O_3 at 196 $\mu g/m^3$ (0.1 ppm) in which the bacterial aerosol was administered either immediately or 2, 4, or 6 hr after cessation of the O_3 exposure. For these postexposure challenges, only the 2-hr time resulted in a significant increase in mortality (6.7 percent) over controls. However, when the animals were infected with streptococci during the actual O_3 exposure, a significant increase in mortality of 21 percent was observed. When this latter experimental regimen was used, exposure to 157 $\mu g/m^3$ (0.08 ppm) of O_3 resulted in a significant increase in mortality of 5.4 percent.

The differences in results among these studies may have been due to a variation in the sensitivity of the method of O_3 monitoring, a difference in mouse strain, changes in the pathogenicity of the bacteria, or differences in sample size. The results from such studies that use this infectivity model indicate the model's sensitivity for detecting biological effects at low pollutant concentrations and its response to modifications in technique (i.e., using different mouse strains or varying the time of bacterial challenge). The model is supported by experimental evidence showing that pollutants (albeit at different concentrations) that cause an enhancement of mortality in the infectivity system also cause reductions in essential host defense systems, such as pulmonary bactericidal capability, the functioning of the alveolar macrophage, and the cytological and biochemical integrity of the alveolar macrophage.

The pulmonary defenses in the O_3-treatment group were significantly less effective in combating the infectious agent to the extent that, even at low concentrations, there were significant increases in mortality over controls. As the O_3 concentration increased, mortality increased. In some studies, additive effects were reported. These effects, increases in respiratory infection, are supported by many mechanistic studies discussed in this section. They indicate that O_3 does effectively cause a reduction in a number of essential host defenses that would normally play a major role in fighting pulmonary infections. Since 1978, a number of new studies have continued to confirm these previous findings and improve the existing data base (Table 9-13).

When mice were exposed 4 hr to 392 to 1372 $\mu g/m^3$ (0.2 to 0.7 ppm) of O_3 and then challenged with virulent _Klebsiella_ _pneumoniae_, a significant increase in mortality was noted at 785 $\mu g/m^3$ (0.4 ppm) O_3 (Bergers et al., 1983). Groups of 30 mice inhaled approximately 30, 100, and 300 bacteria/mouse. At 392 $\mu g/m^3$ (0.2 ppm) of O_3, the O_3 group showed an increase in mortality, but it was not significantly different from controls. At 785 $\mu g/m^3$ (0.4 ppm) of O_3 a nearly threefold lowering of the bacterial LD_{50} value computed from the three challenge doses of bacteria was found for the O_3-exposed group, indicating a significant increase in mortality. In the same study, the authors also found that 1372 $\mu g/m^3$ (0.7 ppm) of O_3 for 3 hr resulted in a decreased ability of the lung to clear (bactericidal) inhaled staphylococci.

TABLE 9-13. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: INTERACTIONS WITH INFECTIOUS AGENTS

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effects	Species	Reference
μg/m³	ppm					
157	0.08	ND	3 hr	Increase in mortality to *Streptococcus* sp.	Mouse	Coffin et al., 1967
157-196	0.08, 0.1	CHEM	3 hr	Significant increase in mortality during O_2 exposure (*Streptococcus* sp.).	Mouse	Miller et al., 1978a
196	0.1	CHEM	3 hr	Increased mortality to *Streptococcus sp*.	Mouse	Ehrlich et al., 1977
196	0.1	UV	5 hr/day, 5 days/wk for 103 days	Increased susceptibility to bacterial infection (*Streptococcus* sp.).	Mouse	Aranyi et al., 1983
196, 588	0.1, 0.3	CHEM	3 hr	Exercise enhances mortality in infectivity model system.	Mouse	Illing et al., 1980
392-1372	0.2-0.7	G	3-4 hr	Significant increase in mortality following challenge with aerosol of *Klebsiella pneumoniae*. Effect seen at 0.4 ppm.	Mouse	Bergers et al., 1983
588	0.3	ND	3 hr/day for 2 days	Enhancement of severity of bacterial pneumonia (*Pasteurella haemolytica*).	Sheep	Abraham et al., 1982
1372-1764	0.7-0.9	ND	3 hr	Increased susceptibility to infection (*Streptococcus* sp.).	Mouse	Coffin and Blommer, 1970
1960	1.0	CHEM	3 hr/day, 5 days/wk for 8 weeks	Increase in *Mycobacterium tuberculosis* lung titers.	Mouse	Thomas et al., 1981b
2940	1.5	ND	4 hr/day, 5 days/wk for 2 months	No effect on resistance to *Mycobacterium tuberculosis*.	Mouse	Thienes et al., 1965
980	0.5	UV	2-4 wks	Reduced widespread viral infection of the lung, resulting in a decrease in disease severity.	Mouse	Wolcott et al., 1982
1254	0.64	CHEM	4 wks	No enhancement of the severity of chronic pulmonary infection with *Pseudomonas aeruginosa*.	Rat	Sherwood et al., 1984

[a] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; UV = UV photometry; G = galvanic meter.

This finding is consistent with that of Goldstein et al. (1971b) and confirms previous studies reported by Miller and Ehrlich (1958) and Ehrlich (1963).

Sherwood et al. (1984) established a chronic pulmonary infection in rats by inoculating agar beads containing viable Pseudomonas aeruginosa (PAO-381) and then exposed these infected animals for 4 wks to 1254 $\mu g/m^3$ (0.64 ppm) O_3. The exposure to ozone did not affect the pulmonary antibacterial defense systems--i.e., no increase in number of organisms cultured from the lung--but it did cause significant anatomical damages. The lungs of rats exposed to O_3 were larger and heavier when compared with controls, and had an increased number of macrophages in their terminal bronchioles. The authors state that the reason why these results are different than those described above for the "infectivity model" is that in these later studies, the infective organisms are given by inhalation and are therefore deposited throughout the lung and the pollutant was able to interfere with the initial phase of the host-parasite interaction. In this chronic study, the infection was isolated to the distal area of the lung and it was in its later stage of development when the animals received the O_3 exposure. Thus, the timing of the exposure to O_3 may be a significant factor in the impairment of the lung's antibacterial defenses.

Exposure to ambient levels of O_3 (0.5 ppm) for 2 to 4 wks has been shown to alter the pathogenesis of respiratory infection of mice with influenza A - virus (Wolcott et al., 1982). The O_3-exposed animals showed a reduction in severity of the disease (less mortality) and an increase in survival time. The reduction of disease severity appears to be dependent on the continuous presence of the O_3 during the infectious process. This effect did not correlate with virus, interferon, or neutralizing antibody titers recovered from the lung, or with neutralizing antibody titers in the sera. The reduced disease severity in the O_3-exposed animals appears to be due to significant alterations in the distribution of viral antigens within the pulmonary tissues, i.e., less widespread infection of the lung.

Chiappino and Vigiani (1982) also reported that O_3 potentiated pulmonary infection in rats. In this study, the investigators wanted to know in what way O_3 modified the reactions to silica in specific pathogen-free (SPF) rats. Silica-treated animals were exposed to 1960 $\mu g/m^3$ (1.0 ppm) O_3 (8 hr/day, 5 days a week for up to 1 year) and housed in either an SPF environment or in a conventional animal house, thus being exposed to the bacterial flora normally present there. The SPF-maintained, O_3-exposed rats showed a complete absence of saprophytes and pathogens in the lungs, whereas the microbial flora of the lungs of the conventionally kept rats, also exposed, consisted of staphylococci (2500 to 4000 per gram of tissue) and streptococci (300 to 800 per gram of tissue). The lungs of these rats showed bronchitis, purulent bronchiolitis, and foci of pneumonia. The exposure to O_3 did not have any effect on particle retention, nor did it modify the lungs' reaction to silica, but it did increase the animals' susceptibility to respiratory infections.

Changes in susceptibility to infection resulting from O_3 exposure have also been tested in sheep. In these studies, sheep were infected by an inoculation of Pasteurella haemolytica either 2 days before being exposed to 588 $\mu g/m^3$ (0.3 ppm) of O_3 or 2 days after the O_3 exposure. In both cases, the O_3 exposure was for 3 hr/day for 2 days. Ozone enhanced the severity of the disease (volume of consolidated lung tissues), with the greatest effect seen when the O_3 insult followed the exposure to the bacteria (Abraham et al., 1982). Unfortunately, only a small number of animals were used in each O_3 treatment (n = 3).

Thomas et al. (1981b) studied the effects of single and multiple exposures to O_3 on the susceptibility of mice to experimental tuberculosis. Multiple exposures to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 3 hr/day, 5 days/week for up to 8 weeks, initiated 7 or 14 days after the infectious challenge with Mycobacterium tuberculosis H37RV, resulted in significantly increased bacterial lung titers, as compared with controls. Exposure to lower concentrations of O_3 did not produce any significant effects. In an earlier study, Thienes et al. (1965) reported that exposure to 2940 $\mu g/m^3$ (1.5 ppm) of O_3 4 hr/day, 5 days/week for 2 months also did not alter the resistance of mice to M. tuberculosis H37RV, but he did not measure lung titers.

Table 9-14 summarizes a number of studies that used mixtures of pollutants in their exposure regimes. Ehrlich et al. (1979) and Ehrlich (1983) expanded the earlier 3-hr-exposure studies to determine the effects of longer periods of exposure to O_3 and NO_2 mixtures. In the earlier studies (Ehrlich et al., 1977), they reported that exposures to mixtures containing 196 to 980 $\mu g/m^3$ (0.1 to 0.5 ppm) of O_3 and 2920 to 9400 $\mu g/m^3$ (1.5 to 5 ppm) of NO_2 produced an additive effect expressed as an increased susceptibility to streptococcal pneumonia. In the later studies, mice were exposed 3 hr/day, 5 days/week for up to 6 months to mixtures of 196 $\mu g/m^3$ (0.1 ppm) of O_3 and 940 $\mu g/m^3$ (0.5 ppm) of NO_2 and challenged with bacterial aerosol. The 1- or 2-month exposure

TABLE 9-14. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: MIXTURES

Ozone concentration µg/m³	Ozone concentration ppm	Pollutant	Measurement[a] method	Exposure duration and protocol	Observed effects[b]	Species	Reference
98 3760	0.05	O_3 + NO_2	CHEM	3 hr	Exposure to mixtures caused synergistic effect after multiple exposures; additive effect after single exposure.	Mouse	Ehrlich et al., 1977, 1979, 1980
98-196 100-400 1500	0.05-0.1	O_3 + NO_2 + $ZnSO_4$	CHEM	3 hr	Additive effect of pollutant mixtures with infectivity model.	Mouse	Ehrlich, 1983
196 241-483	0.1	O_3 + H_2SO_4	CHEM	3 hr	Increased susceptibility to Streptococcus pyogenes.	Mouse	Grose et al., 1982
196 900	0.1	O_3 + H_2SO_4	CHEM	3 hr + 2 hr	Sequential exposure resulted in significant increase in respiratory infection. Neither alone produced a significant effect.	Mouse	Gardner et al., 1977
196 1090	0.1	O_3 + H_2SO_4	CHEM	3 hr + 2 hr	Sequential exposure resulted in significant reduction in ciliary beating activity over H_2SO_4 alone.	Hamster	Grose et al., 1980
196 940	0.1	O_3 + NO_2	CHEM	3 months	Significant decrease in viability of alveolar macrophages seen with mixtures.	Mouse	Ehrlich et al., 1979
196 940	0.1	O_3 + NO_2	CHEM	3 hr/day for 1-6 months	At 3 and 6 months, susceptibility to pulmonary infection increased significantly. Delayed clearance rate.	Mouse	Ehrlich, 1980, 1983
196 13200 1040	0.1	O_3 + SO_2 + $(NH_4)_2SO_4$	UV	5 hr/day, 5 days/wk for 103 days	Highly significant increase in susceptibility to infection. Effects attributed to O_3. Increased bactericidal rate over O_3 alone. Mixture showed greater growth inhibition in leukemia target cells and an increase in blastogenic response to PHA, Con-A, and alloantigens.	Mouse	Aranyi et al., 1983

TABLE 9-14. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: MIXTURES (continued)

Ozone concentration		Pollutant	Measurement[a] method	Exposure duration and protocol	Observed effects[b]	Species	Reference
μg/m³	ppm						
216-784 3760-13720	0.11-0.4 2-7.3	O_3 + NO_2	A	17 hr before bacteria or 4 hr after bacterial exposure	Physical removal of bacteria not affected. Bactericidal activity reduced at higher concentrations.	Mouse	Goldstein, E., et al., 1974
980 11-3000	0.5	O_3 + H_2SO_4	UV	3 and 14 days	Significant increase in glycoprotein secretion; synergism reported; effect reversible in clean air.	Rat	Last and Cross, 1978
1570 3500 3500 3500	0.8	O_3 + $Fe_2(SO_4)_3$ + H_2SO_4 + $(NH_4)_2SO_4$	UV	4 hr	Exposure to mixtures produced same effect as exposure to O_3 alone.	Rat	Phalen et al., 1980

[a] Measurement method: CHEM = gas phase chemiluminescence; UV = UV photometry; A = amperometric.

[b] Abbreviations used: PHA = phytohemagglutinin; Con-A = concanavalin-A

did not induce any significant changes in susceptibility to streptococcal infection. After 3 and 6 months of exposure, the resistance to infection was significantly reduced. If the mice were re-exposed to the O_3 and NO_2 mixture after the infectious challenge, a significant increase in mortality rate could be detected 1 month earlier. The clearance rate of inhaled viable streptococci from the lungs also became significantly slower after the 3-month exposure to this oxidant mixture.

In more complex exposure studies, mice were exposed to a background concentration of 188 $\mu g/m^3$ (0.1 ppm) of NO_2 for 24 hr/day, 7 days/week with a superimposed 3-hr daily peak (5 days/week) containing a mixture of 196 $\mu g/m^3$ (0.1 ppm) of O_3 and 940 $\mu g/m^3$ (0.5 ppm) of NO_2. Mortality rates from streptococcal infection were not altered by 1- and 2-month exposures, but a marked, although only marginally significant ($p < 0.1$), increase was seen after a 6-month exposure.

The same laboratory (Aranyi et al., 1983) recently reported a study in which mice were exposed 5 hr daily, 5 days/week up to 103 days to O_3 and a mixture of O_3, SO_2, and $(NH_4)_2SO_4$. The concentrations were 196 $\mu g/m^3$ (0.1 ppm) of O_3, 13.2 mg/m^3 of SO_2, and 1.04 mg/m^3 of $(NH_4)_2SO_4$. Both groups showed a highly significant overall increase in mortality, compared to control mice exposed to filtered air. However, the two exposure groups did not differ, indicating that O_3 was the major constituent of the mixture affecting the host's susceptibility to infection. These investigators also measured a number of specific host defenses to determine if host response to the mixtures was significantly different from that of the controls or of the O_3-only groups. Neither the total number, differential, nor the ATP levels of macrophages differed from controls in either group, but the complex mixture did produce a significant increase in bactericidal activity over the O_3-alone and the control animals.

Previous studies (Gardner et al., 1977) indicated that a sequential exposure to 196 $\mu g/m^3$ (0.1 ppm) O_3 followed by 1000 $\mu g/m^3$ H_2SO_4 significantly increased streptococcal pneumonia-induced mortality rates in mice. Ozone and H_2SO_4 had an additive effect when exposed in this sequence. However, the reverse sequence did not affect incidence of mortality. Sulfuric acid alone caused no significant effect. Grose et al. (1982) expanded these studies and showed that a 3-hr exposure to the mixture of 196 $\mu g/m^3$ (0.1 ppm) of O_3 and

483 or 241 μg/m^3 H_2SO_4 also significantly increased the percent mortality, as compared to control.

The effects of exercise on the response to low levels of O_3 were also studied by using the infectivity model. Mice were exposed to 196 μg/m^3 (0.1 ppm) of O_3 and 588 μg/m^3 (0.3 ppm) of O_3 for 3 hr while exercising. Each exposure level yielded mortality rates that were significantly higher than those observed in the O_3 group that was not exercised (Illing et al., 1980). Such activity could change pulmonary dosimetry, thus increasing the amount of O_3 reaching the respiratory system. Thus, such studies clearly demonstrate that the activity level of the exposed subjects is an important concomitant variable influencing the determination of the lowest effective concentration of the pollutant.

9.3.4.4 Immunology. In addition to the above nonspecific, nonselective mechanisms of pulmonary defense, the respiratory system also is provided with specific, immunologic mechanisms, which can be activated by inhaled antigens. There are two types of immune mechanisms: antibody (humoral)-mediated and cell-mediated. Both serve to protect the respiratory tract against inhaled pathogens. Much less information is available on how O_3 reacts with these immunological defenses than is known about the macrophage system (see Table 9-15). Most studies have involved the systemic immune system which, to a degree, is compartmentalized from the pulmonary immune system.

The effects of 2900 μg/m^3 (1.48 ppm) of O_3 for 3 hr on cell-mediated immune response were studied by Thomas et al. (1981b), who determined the cutaneous delayed hypersensitivity reaction to purified protein derivative (PPD), expressed as the diameter of erythemas. In the guinea pigs infected with inhaled *Mycobacterium tuberculosis*, the cutaneous sensitivity to PPD was significantly affected by O_3. The diameters of the erythemas from the O_3-exposed animals were significantly smaller during the 4 to 7 weeks after the infectious challenge, indicating depressed cell-mediated immune response. Exposure to 980 μg/m^3 (0.5 ppm) of O_3 had no effect.

The systemic immune system was studied by Aranyi et al. (1983), who observed the blastogenic response of splenic lymphocytes to mitogens and alloantigens and plaque-forming cells' response to sheep red blood cells after a chronic exposure to 196 μg/m^3 (0.1 ppm) of O_3 5 hr/day, 5 days/week for 90 days. No alteration in the response to alloantigens or the B-cell mitogen lipopolysaccharide (LPS) was noted, but a statistically significant suppression in

TABLE 9-15. EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS: IMMUNOLOGY

Ozone concentration μg/m^3	Ozone concentration ppm	Measurement[a] method	Exposure duration and protocol	Observed effects[b]	Species	Reference
196	0.1	UV	5 hr/day, 5 days/wk for 90 days	Significant suppression in blastogenesis to T-cell mitogen, PHA, and Con-A. No effect on B-cell mitogen, LPS, or alloantigen of splenic lymphocytes.	Mouse	Aranyi et al., 1983
980, 2940	0.5, 1.5	ND	4 hr	Attempt to increase immune activity with drug Levamisole failed.	Mouse	Goldstein et al., 1978a
980, 1568	0.5, 0.8	Mast	Continuous 3-4 days	Increase in number of IgE- and IgA-containing cells in the lung, resulting in an increase in anaphylactic sensitivity.	Mouse	Osebold et al., 1979, 1980 Gershwin et al., 1981
980-2900	0.5-1.48	CHEM	3 hr	Depressed cell-mediated immunity. No effect at 0.5 ppm for 5 days. Hemagglutination antibody titers increased over control.	Guinea pig	Thomas et al., 1981b
1150	0.59	ND	36 days	Impaired resistance to toxin stress. Immunosuppression.	Mouse	Campbell and Hilsenroth, 1976
1568	0.8	ND	1, 3, 7, and 14 days	Depressed splenic lymphocyte response to T-cell dependent antigen that correlated with changes in thymus weights.	Mouse	Fujimaki et al., 1984

[a] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; UV = UV photometry; Mast = Mast meter.

[b] Abbreviations used: PHA = phytohemagglutinin; con-A = concanavalin-A; LPS = lipopolysaccharide; IgE = immunoglobulin-E; IgA = immunoglobulin-A.

blastogenesis to the T-cell mitogens (PHA and Con-A) was detected. The authors suggested that the cellular mechanisms for recognition and proliferation to LPS and alloantigens were intact, but O_3 might have been interfering with the cellular response to PHA and Con-A through alterations in cell surface receptors or the binding of specific mitogens to them. Ozone exposure enhances peritoneal macrophage cytostasis to tumor target cells. There was no effect on the ability of splenic lymphocytes to produce antibodies against injected antigen (red blood cells).

The effects of O_3 on the humoral immune response was also studied by Fujimaki et al. (1984). BALB/c mice that were exposed continuously to 1568 $\mu g/m^3$ (0.8 ppm) had an increase in lung weight after 3, 7, and 14 days of exposure. Spleen weights were decreased only after 1 and 3 days of exposure and then returned to normal. The thymus weight was decreased at all time periods tested, i.e., 1, 3, 7, and 14 days. Similar mice exposed to 0.4 ppm failed to show any marked changes in the organ weights. At 0.8 ppm O_3, exposure depressed the antibody response to sheep red blood cells (T-cell dependent antigen), but not at the antibody response to the T-cell independent antigen. These changes were correlated with the changes of thymus weights. The authors also concluded that O_3 affected mainly the T-cell population rather than the B-cell population.

Campbell and Hilsenroth (1976) used a toxoid immunization-toxin challenge approach to determine if continuous exposure to 1150 $\mu g/m^3$ (0.59 ppm) of O_3 for 36 days impaired resistance to a toxin stress. Mice were immunized with tetanus toxoid on the fifth day of O_3 exposure and challenged with the tetanus toxin on day 27. Compared with controls, the O_3-exposed animals had greater mortality and morbidity following the challenge. The authors suggested that the effect was due to immunosuppression.

Because the data indicate that O_3 can alter the functioning of pulmonary macrophages, Goldstein et al. (1978a) tried to counteract this effect by using a known immunologic stimulant, Levamisole, which protects rodents against systemic infections of staphylococci and streptococci. The purpose was to determine if this drug might repair this dysfunction and improve the bactericidal activity of the macrophage. In this study, two concentrations were tested, 2940 and 980 $\mu g/m^3$ (0.5 and 1.5 ppm) of O_3 for 4 hr. In no case did Levamisole improve the bactericidal activity of the O_3-exposed macrophages. The cells still failed to respond normally.

The possibility that O_3 may be responsible for the enhancement of allergic sensitization has important implications for human health effects. Gershwin et al. (1981) reported that O_3 (0.8 and 0.5 ppm for 4 days) exposure caused a thirty-fourfold increase in the number of IgE-containing cells in the lungs of mice that had been previously exposed to aerosolized ovalbumin. In general, the number of IgE-containing cells correlated positively with levels of anaphylactic sensitivity. Oxidant damage (0.5 to 0.8 ppm for 4 days) also causes an increase in IgA-containing cells in the lungs and a rise in IgA content in respiratory secretions and accumulation of lymphoid tissue along the airways (Osebold et al., 1979). The number of IgM and IgG containing cells did not increase. These authors showed that a significant increase in anaphylactic sensitivity occurred when antigen-stimulated and O_3-exposed animals were compared to controls (Osebold et al., 1980). Significantly greater numbers of animals were allergic in experimental groups when O_3 exposure ranged from 0.8 ppm to 0.5 ppm for 3 days. The effects observed were most pronounced when the allergen (ovalbumin) was administered by I.V. injection than by the aerosol route. Further studies are needed to determine the threshold level of these effects.

9.3.5 Tolerance

Acclimatization, whether it be a long-term or a moment-to-moment response of the organism to a changing environment, has been a phenomenon of major interest to toxicologists for years. Tolerance, in the broadest sense of the word, may be viewed as a special form of acclimatization in which exposure to a chemical agent results in increased resistance, either partial or complete, to the toxicant (Hammond and Beliles, 1980). Often the terms tolerance and resistance are used interchangeably. The word, tolerance, is primarily used when the observed decrease in susceptibility occurs in an individual organism as a result of its own previous or continuing exposure to the particular toxicant or to some other related stimulus. Resistance generally refers to relative insusceptibility that is genetically determined (Hayes, 1975).

A third term, adaptation, has been widely used primarily to describe the diminution of response seen in human subjects who have undergone repeated O_3 exposure (Chapter 10, Section 3). This adaptation might well result from a different biologic process than that referred to in the various animal tolerance studies. It is not yet known whether the laboratory animal develops adaptive responses similar to those seen in humans (i.e., respiratory mechanical functions, symptoms of respiratory irritation, and airway reactivity). Thus, to date, the precise distinction or definition of these two terms, tolerance and adaptation, are not yet fully understood. There are also limited data regarding the ability of cells in these "adapted" respiratory systems to once again return to a pre-exposure condition following termination of the exposure. However, Plopper et al. (1978) found that after 6 days in clean air, the rat's lungs were almost recovered from the damage caused by 3 days of exposure to 0.8 ppm O_3 and that these "new recovered" cells have approximately the same degree of susceptibility to a re-exposure of O_3 as their pre-exposed counterparts, i.e., fully susceptible to re-exposure. These morphological findings confirm similar conclusions based on concomitant biochemical studies of Chow et al. (1976b).

In animal oxidant toxicity studies, the term tolerance classically is defined as the phenomenon wherein a previous exposure to a nonlethal concentration of O_3 will provide some protection against a subsequent exposure to a concentration of O_3 expected to be lethal. The degree of tolerance depends considerably on the duration of the exposure and the concentration. Tolerance occurs rapidly and can persist for several weeks (Mustafa and Tierney, 1978). The term tolerance should not be considered to indicate complete or absolute protection, because continuing injury does occur and can eventually lead to nonreversible morphological changes. This protective phenomenon seen with oxidants was originally described by Laqueur and Magnus (1921) in cats undergoing exposure to phosgene.

In the typical experiment, animals are pre-exposed to a lower concentration of O_3 and then challenged at a later time to a higher concentration. As early as 1956, Stokinger et al. presented data clearly indicating that an animal could also become tolerant to the lethal effects of O_3. Such tolerance has also been reported by many investigators, including Matzen (1957a), Mendenhall and Stokinger (1959), Henschler (1960), and Fairchild (1967). The observation of this tolerance phenomenon in experimentally exposed animals has led to the speculation that it may also be a mechanism for protecting environmentally exposed humans. Tolerance to O_3 also provides cross-protection against the pulmonary effects of other chemical agents, such as NO_2, ketene, phosgene, and hydrogen peroxide (Stokinger and Coffin, 1968) and recently to hyperoxia (Jackson and Frank, 1984).

The previous criteria document for O_3 and other photochemical oxidants cited various studies that examined O_3 tolerance and presented some evidence indicating possible mechanisms of action. Review of these earlier data reveals that pre-exposure to a certain concentration of O_3 can protect test animals from the acute lethal effects of a second exposure to O_3. This protection has been attributed to a significant reduction in pulmonary edema in the pre-exposed animals. Table 9-16 lists the key studies on O_3 tolerance.

Because O_3 has a marked proclivity to reduce the ability of alveolar macrophages to function, studies were conducted to determine how the pulmonary defense system in tolerant animals compared with naive animals. With the bacterial infectivity model (Section 9.3.4.3), the pre-exposed (tolerant) animals were only partially protected from the aerosol infectious challenge (Coffin and Gardner, 1972a; Gardner and Graham, 1977). The partial protection was evident at O_3 concentrations that had been shown to be edemagenic; however, at the lowest concentration, 200 $\mu g/m^3$ (0.10 ppm) of O_3, there was no significant difference imparted by the use of the tolerant-eliciting exposure. The data suggest that at the higher concentrations (> 0.3 ppm), pre-exposure prevented edema, which prophylactically aided the animals' defenses against the inhaled microorganisms. Because the protection was only fractional and did not occur at the lowest level, however, O_3 still suppressed specific body defenses that were not protected by the phenomenon of tolerance.

To further investigate this hypothesis (Alpert and Lewis, 1971; Gardner et al., 1972), studies were conducted to evaluate the effects of tolerance at the cellular level. These studies indicated that the initial O_3 exposure did induce tolerance against pulmonary edema in the exposed lung; however, there was no protection afforded against the cytotoxic effects of O_3 at the cellular level. The cytological toxic injuries measured in this study (including significant reductions in enzymatic activities of macrophages and an increase in inflammation, as measured by the presence of polymorphonuclear leukocytes) showed that there was no protection against these cellular defense mechanisms.

Frager et al. (1979) studied the possibility of tolerance to O_3 in mucociliary clearance. Exposure of rats to 1.2 ppm of O_3 following particle deposition caused a substantial delay in mucociliary clearance. The O_3 effect could be eliminated by a pre-exposure to 1600 $\mu g/m^3$ (0.80 ppm) of O_3 for 4 hr, 3 days before the deposition of the particles. Thus, the pre-exposure provided complete protection against the higher O_3 level that lasted for about one

TABLE 9-16. TOLERANCE TO OZONE

Ozone ($\mu g/m^3$) pre-exposure	Ozone (ppm) pre-exposure	Length of pre-exposure	Ozone ($\mu g/m^3$) after latent period	Ozone (ppm) after latent period	Length of exposure after latent period	Observed effect(s)	Species	Reference
196-1960	0.1-1.0	3 hr	196-1960	0.1-1.0	3 hr	Lower mortality for pre-exposed mice than mice receiving only one O_3 dose. Complete tolerance was not evident.	Mouse	Gardner and Graham, 1977
196	0.1	30 min	196	0.1	30 min	Tolerance exhibited in the lungs' periphery, as measured by collateral resistance. Response ≤ controls in tolerant animals.	Dog	Gertner et al., 1983b
490	0.25	6 hr	1966	1	6 hr	No tolerance to edema unless pretreated with methylprednisolone.	Rat	Alpert et al., 1971a
980	0.5	6 hr				Edema as measured by recovery of ^{132}I in pulmonary lavage fluid.		
588	0.3	1 hr	39,200	20	2 hr	Tolerance to edema effects of O_3 did not develop in thymectomized animals but developed in sham-operated animals, indicating the thymus may be involved in tolerance.	Mouse	Gregory et al., 1967
588	0.3	3 hr	588	0.3	3 hr	20% lower mortality for pre-exposed mice than mice receiving only one O_3 dose. Partial tolerance probably due to inhibition of edemagenesis.	Mouse	Coffin and Gardner, 1972a
588-980	0.3-0.5	4 days	980 1372 1960	0.5 0.7 1.0	1, 2, 4 days	Lack of total protection indicated by increased numbers of type 2 cells.	Rat	Evans et al., 1971, 1976a,b
980-1960	0.5, 1.0	3 hr	43,120	22	3 hr	With unilateral lung exposure technique, tolerance to edema occurred as a local effect (cellular) and was seen only in the pre-exposed lung.	Rabbit	Alpert et al., 1971b Alpert and Lewis, 1971

TABLE 9-16. TOLERANCE TO OZONE (continued)

Ozone ($\mu g/m^3$) pre-exposure	Ozone (ppm) pre-exposure	Length of pre-exposure	Ozone ($\mu g/m^3$) after latent period	Ozone (ppm) after latent period	Length of exposure after latent period	Observed effect(s)	Species	Reference
980	0.5	3 hr	5880 or	3 and 22	3 hr	With unilateral lung exposure technique, tolerance developed only to pulmonary edema. No tolerance to the chemotaxis of polymorphonuclear leukocytes or decreased lysosomal hydrolase enzyme activity.	Rabbit	Gardner et al., 1972
1470	0.75	3 days	7840	4.0	8 hr	A smaller decrease in activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and levels of reduced glutathione in lungs of tolerant animals, as compared to nontolerant animals.	Rat	Chow, 1976 Chow et al., 1976b
1490	0.76	3 day	6860-7840	3.5-4	8 hr	When latent period was 11 days, no tolerance to decrease in GSH peroxidase system immediately after challenge; 18 hr later, a smaller decrease occurred. When latent period was 19 days, the decrease in enzyme activities measured 16 hr post-challenge was less in pre-exposed animals; 114 hr post-challenge, some increases in the GSH peroxidase system were observed.	Rat	Chow, 1984
1570	0.8	3 days	1570	0.8	6 or 27 days	After 6 days of recovery the lung is again fully susceptible to re-exposure. Adaptation lasts only as long as the O_3 exposure continues.	Rat	Plopper et al., 1978
1600	0.8	4 hr	2352	1.2	4 hr	Pre-exposure to O_3 caused complete tolerance to delay in mucociliary clearance at 3 days, but not 13 days.	Rat	Frager et al., 1979
1960	1	1 hr	ND	ND	ND	All animals X-irradiated to 800 R. 60% of O_3-pre-exposed mice survived. 100% of controls died.	Mouse	Hattori et al., 1963
1960	1	1 hr	3920	2	1 hr	Tolerance to allergic response to inhaled acetylcholine.	Guinea pig	Matsumura et al., 1972

ND = not described.

week. The possible mechanism for this protection could be a thickening of the mucus layer, which would offer the epithelium an extra physical barrier against O_3. As the secretion returns to normal, the protection is lost. The authors suggested that another possible mechanism for this protection involves the ciliated cells and their cilia. In this case, the protection could result from either the formation of intermediate cilia (Hilding and Hilding, 1966) or the occurrence of some other temporary change in the regenerating ciliated cell.

Evans et al. (1971, 1976b) also measured tolerance by studying the kinetics of alveolar cell division in rats during a period of exposure to an elevated O_3 concentration of 980 or 1372 $\mu g/m^3$ (0.50 or 0.70 ppm, up to four days) that followed initial exposure at a lower concentration of 686 $\mu g/m^3$ (0.35 ppm) for four days. Tolerance in this case was the ability of type 1 cells to withstand a second exposure without any increase in the number of type 2 cells, which would indicate a lack of complete tolerance. Similar to the host defense studies cited above, these investigations showed that tolerance to the initial concentration of O_3 did not ensure complete protection against re-exposure to the higher O_3 concentration.

Attempts have been made to explain tolerance by examining the morphological changes that occur due to repeated exposures to O_3. In these studies the investigators attempt to assess various structural responses with various exposure profiles and concentrations. Dungworth et al. (1975b) and Castleman et al. (1980) studied the repair rate of O_3 damage as indicated by DNA synthesis. These effects are fully described in Section 9.3.1.2, and they indicate that with continuous exposure to O_3, the lung attempts to initiate the repair of the O_3 lesion, resulting in somewhat reduced or less than expected total damage. These authors suggest that this is an indication that although the damage is continuing, it is at a lower rate, and they refer to this phenomenon as adaptation.

It has been suggested that the tolerance to edema seen in animal studies can be explained through the indirect evidence that more resistant cells, such as type 2 cells, may replace the more sensitive, older type 1 cells, or that the type 2 cells may transform to younger, more resistant cells of the same type (Mustafa and Tierney, 1978). A number of workers have reported that the younger type 1 cells are relatively more resistant to the subsequent toxicity of O_3 (Evans et al., 1976a; Dungworth et al., 1975a; Schwartz et al., 1976).

Thus, there is also the possibility that this reparative-proliferative response relines the airway epithelium with cells that have a biochemical armamentarium more resistant to oxidative stress (Mustafa et al., 1977; Mustafa and Lee, 1976).

Another suggestion is that with O_3 exposure, there is cellular accumulation within the airways resulting in mounds of cells in the terminal bronchioles that may cause considerable narrowing of the airways (Berliner et al., 1978). As the airways become more obstructed, the O_3 molecules are less likely to penetrate to lumen. This may result in a "filtering" system that removes the O_3 before it reaches the sensitive tissue.

Tolerance to O_3 has also been studied by using a variety of biochemical indicators to measure the extent to which a pre-exposure to O_3 protects or reduces the host response to a subsequent exposure. For example, Jackson and Frank (1984) found that preexposure to O_3 produced cross tolerance to hypoxia. In these tolerant animals (0.8 ppm O_3 x 7 days) there was a significant increase in total lung superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, and catalase. Such an increase in these antioxidant enzymes occurs with O_3 exposure. Chow et al. (1976a,b) compared a variety of metabolic activities of the lung immediately after an initial 3-day continuous exposure to 1600 $\mu g/m^3$ (0.80 ppm) of O_3 with the response after subsequent re-exposure. At 6, 13, and 27 days after the pre-exposure ended, the animals were once again treated to the same exposure routine. If tolerant, the animals should have shown a diminution of response. However, the re-exposed rats responded similarly to those animals tested after the initial exposure. The lungs of the naive animals had equivalently higher activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and higher levels of nonprotein sulfhydryl than controls and were comparable to the animals that were exposed and tested immediately after the initial exposure. The authors state that this indicated that by the time recovery from the pre-exposure is complete, the lung is as susceptible to the re-exposure injury as a lung that has never been exposed.

In a follow-up study, Chow (1984) pre-exposed rats for 3 days (apparently continuously) to air or 1490 $\mu g/m^3$ (0.76 ppm) O_3 and challenged them at various times with an 8-hr exposure to a higher level of O_3. As expected, the pre-exposure protected the rats from the lethal effects of 6860 to 7840 $\mu g/m^3$ (3.5 to 4.0 ppm) O_3, whether the challenge was 8, 11, or 19 days later. Generally, all O_3 exposures decreased the GSH peroxidase system. When rats were challenged with 7644 μg/m^3 (3.9 ppm) O_3 11 days after the 3-day pre-exposure, there was no tolerance immediately after the challenge exposure; 18 hr after the challenge exposure, a dampening of the decrease in the GSH peroxidase system was observed. When the pre-exposure and challenge (6860 μg/m^3, 3.5 ppm) were separated by 19 days, the decrease in the GSH peroxidase system measured 16 hr post-challenge was less in the animals receiving pre-exposure (except for GSH reductase, for which values were equivalent); 114 hr post-challenge, no tolerance was observed, but some increases in enzyme activity were observed.

Gertner et al. (1983b) presented data showing that the development of adaptation and tolerance to pulmonary function changes is rapid and mediated through the vagus nerve. These investigators used a bronchoscope to expose an isolated segmental airway of the lung to O_3 and study changes in collateral resistance (Rcoll). During a 30-min exposure to 0.1 ppm, the Rcoll increased 31.5 percent within 2 min and then gradually decreased to control level in spite of continual exposure to O_3. Fifteen minutes after the O_3 exposure ceased, the Rcoll returned to normal. Subsequent exposure to 0.1 ppm of O_3 did not increase Rcoll, indicating that some protection existed. These investigators have tried to distinguish between the terms adaptation and tolerance based on these studies. They used adaptation to describe the pattern of changes that occur during continuous exposure to O_3 and the term tolerance to describe resistance to subsequent O_3 exposure.

Thus, the available evidence from animal studies suggests that tolerance does not develop to all forms of lung injury. The protection described against edemagenic effects of O_3 does not appear to offer complete protection, as illustrated by the following examples.

1. There is no tolerance (i.e., no protection occurs) on the part of the specific pulmonary defense mechanisms against bacterial infection below the edemagenic concentration; whereas above the edema-inducing concentration the effect of tolerance (i.e., inhibition of pulmonary edema), can lower the expected mortality rate because the animals do not have to cope with the additional burden of the edema fluid.
2. Specific cellular functions of the alveolar macrophage (i.e., enzyme activity) are incapable of being protected by tolerance.

3. Various biochemical responses were found in both naive and pre-exposed animals.
4. Tolerance fails to inhibit the influx of polymorphonuclear leukocytes into the airway.

This last finding is interesting considering the effective tolerance for edema production. This suggests that the chemotactic effect of O_3 may be separable from the edemagenic effect. This may also explain why chronic morphological changes in the lung may occur after long-term exposure, even though there may not be any edema.

The possible explanations for this tolerance phenomenon have been proposed by Mustafa and Tierney (1978). The primary mechanism of tolerance may not be due to hormonal or neurogenic pathways, because unilateral lung exposure does not result in tolerance of the nonpre-exposed lung (Gardner et al., 1972; Alpert and Lewis, 1971). But it should be noted that Gertner et al. (1983b) have evidence that local tolerance may involve a neural reflex. Changes in Rcoll may be mediated through the vagus nerve. After bilateral cervical vagotomy, the resistance did not increase during O_3 exposure but did after challenging with histamine, indicating that the parasympathetic system may play a role in response to O_3 in the periphery of the lung. There is some evidence that O_3 may cause a decrease in cellular sensitivity, an increased capacity to destroy the test chemical, or the repair of the injured tissue (Mustafa and Tierney, 1978). In addition, O_3 could possibly cause anatomic changes, such as an increase in mucus thickness, that may, in effect, reduce the dose of O_3 reaching the gas-exchange areas of the lung.

It should be mentioned that the term tolerance carries with it the connotation that some form of an insult and/or damage has occurred and there has been an overt response at the structural and/or functional level. The response may be attenuated or undetectable, but the basis for the establishment of the tolerance still persists. It is possible that the cost for tolerance may be minor, such as a slight increase in mucus secretion; however, one must also be aware that changes in response to diverse kinds of insults to a host's system, such as the immune system, are adaptations that might even suggest an undesirable effect of ambient oxidant air pollution.

9.4 EXTRAPULMONARY EFFECTS OF OZONE

9.4.1 Central Nervous System and Behavioral Effects

Despite reports of headache, dizziness, and irritation of the nose, throat, and chest in humans exposed to O_3 (see Chapter 10), and the possible implications of these and other symptoms as indications of low-level O_3 effects, few recent reports were found on behavioral and other central nervous system (CNS) effects of O_3 exposure in animals. Table 9-17 summarizes studies on avoidance and conditioned behavior, motor activity, and CNS effects.

Early investigations have reported effects of O_3 on behavior patterns in animals. Peterson and Andrews (1963) attempted to characterize the avoidance behavior of mice to O_3 by measuring their reaction to a 30-min exposure on one side of an annular plastic mouse chamber. A concentration-related avoidance of the O_3 side was reported at 1176 to 16,660 $\mu g/m^3$ (0.60 to 8.50 ppm) of O_3. However, the study had serious shortcomings, including a lack of position-reversal controls (Wood, 1979), considerable intersubject variability, and other design flaws (Doty, 1975). Tepper et al. (1983) expanded on the design by using inhalant escape behavior to assess directly the aversive properties of O_3. Mice were individually exposed to O_3 for a maximum of 60 sec, followed by a chamber washout period of 60 sec. The animals could terminate exposure by poking their noses into only one of two brass conical recesses containing a photobeam. The delivery of 980 $\mu g/m^3$ (0.50 ppm) of O_3 was reliably turned off for a greater proportion of experimental trials, compared to control trials with filtered air. At 19,600 $\mu g/m^3$ (10 ppm) of O_3, all animals turned off 100 percent of the trials with an average latency of approximately 10 sec.

Studies by Murphy et al. (1964) demonstrated that wheel-running activity decreased by approximately 50 percent when mice were exposed to 392 to 980 $\mu g/m^3$ (0.20 to 0.50 ppm) of O_3 for 6 hr and decreased to 60 percent of pre-exposure values during the first 2 days of continuous exposure to 588 $\mu g/m^3$ (0.30 ppm) of O_3. Running activity gradually returned to pre-exposure values during the next 5 days of continuous exposure to 588 $\mu g/m^3$ (0.30 ppm) of O_3. If the same mice were subsequently exposed to 1372 $\mu g/m^3$ (0.70 ppm) of O_3 for an additional 7 days, running activity was depressed to 20 percent of pre-exposure values. Partial recovery was described during the final days of exposure to 1372 $\mu g/m^3$ (0.70 ppm), and complete recovery occurred several days after exposure was terminated. However, partial tolerance was seen when the air-control mice were subsequently exposed to 392 $\mu g/m^3$ (0.20 ppm) of O_3 for 7 days. Konigsberg and Bachman (1970) used a capacitance-sensing device to record the motor

TABLE 9-17. CENTRAL NERVOUS SYSTEM AND BEHAVIORAL EFFECTS OF OZONE

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effects(s)[c]	Species	Reference
µg/m³	ppm					
110	0.056	d	93 days, continuous	No overt behavioral changes. Cholinesterase activity inhibited at 75 days of exposure, returning to control levels 12 days after termination of exposure.	Rat	Eglite, 1968
98-1960	0.05-1.0	MAST	45 min	Motor activity progressively decreased with increasing O_3 concentrations up to 0.5 ppm. Slight increase in frequency of 3-min intervals without motor activity.	Rat	Konigsberg and Bachman, 1970
196-3920	0.1-2.0	CHEM	6 hr	Linear and/or monotonic decreases in operant behavior during exposure.	Rat	Weiss et al., 1981
235-1960	0.12-1.0	CHEM	6 hr	Wheel running activity decreased monotonically with increasing O_3 concentration. Components of running were differentially affected at low vs. high O_3 concentrations.	Rat	Tepper et al., 1982
392-980	0.2-0.5	NBKI	6 hr	Wheel running activity decreased 50%.	Mouse	Murphy et al., 1964
588-1372	0.3, 0.7	MAST	7 days, continuous	Running activity decreased 60% during first 2 days, returning to control levels during the next 5 days of exposure; running activity decreased 20% when 0.3 ppm exposure was followed immediately by 0.7 ppm O_3 exposure. Adaptation with continued exposure was apparent.		
980-19600	0.5-10.0	CHEM	60 s	Exposure terminated by nose pokes with increasing frequency as O_3 concentration increased.	Mouse	Tepper et al., 1983
980	0.5	ND	30 min	Elevation of simple and choice reactive time.	Nonhuman primate	Reynolds and Chaffee, 1970

TABLE 9-17. CENTRAL NERVOUS SYSTEM AND BEHAVIORAL EFFECTS OF OZONE (continued)

Ozone concentration		Measurement[a,b] method	Exposure duration and protocol	Observed effects(s)[c]	Species	Reference
μg/m³	ppm					
980-1960	0.5, 1.0	NBKI	1 hr	Evoked response to light flashes in the visual cortex and superior collicus decreased after exposure.	Rat	Xintaras et al., 1966
1176-16,660	0.6-8.5	e	30 min	Avoidance behavior increased with increasing O_3 concentration.	Mouse	Peterson and Andrews, 1963
1960	1.0	MAST NBKI	7 days, continuous	Reduction in wheel running activity; no effect of Vitamin E deficiency or supplementation.	Rat	Fletcher and Tappel, 1973
1960-5880	1-3	ND	18 months, 8 hr/day	COMT activity decreased at 2 ppm, MAO activity increased at 1 ppm only.	Dog	Trams et al., 1972
1960	1		18 months; 8, 6, 24 hr/day	COMT activity decreased as the daily exposure increased from 8 to 24 hr. MAO activity increased at 8 and 16 hr/day and decreased at 24 hr/day.		
1960-5880	1-3	ND	18 months, 8 hr/day	Alterations in EEG patterns after 9 months, but not after 18 months of exposure.	Dog	Johnson et al., 1976

[a] Measurement method: MAST = KI-coulometric (Mast meter); CHEM = gas phase chemiluminescence; NBKI = neutral buffered potassium iodide; ND = not described.

[b] Calibration method: NBKI = neutral buffered potassium iodide.

[c] Abbreviations used: COMT = catechol-o-methyltransferase; MAO = monamine oxidase; EEG = electroencephalogram

[d] Spectrophotometric method with dihydroacridine.

[e] KI titration with sodium thiosulfate.

activity of rats during a 45-min exposure to 98, 196, 392, 980, and 1960 μg/m^3 (0.05, 0.10, 0.20, 0.50, and 1.0 ppm) of O_3. Compared with control rats, motor activity following O_3 exposure progressively decreased with increasing O_3 concentrations up to 980 μg/m^3 (0.50 ppm). No greater reduction was obtained at 1960 μg/m^3 (1.0 ppm). In addition, the frequency of 3-min intervals without measurable motor activity tended to increase slightly (from 1 to 1.25 to approximately 3) with increasing O_3 concentration.

A detailed microanalysis of motor activity was undertaken by Tepper et al. (1982), who exposed rats for 6-hr periods during the nocturnal phase of their light-dark cycle to 235, 490, 980, and 1960 μg/m^3 (0.12, 0.25, 0.50 and 1.0 ppm) of O_3. The 3 days preceding an exposure were used for control observations to measure running activity for each rat in a wheel attached to the home cage. Decreases in wheel running activity occurred at 235 μg/m^3 (0.12 ppm) and progressively greater decreases in wheel running activity occurred with increasing O_3 concentration. An analysis of the running behavior showed that the components of running were differentially affected by O_3. An increase in the time interval between running bursts primarily accounted for the decreased motor activity at the low (235 μg/m^3, 0.12 ppm) O_3 concentration. Postexposure increases in wheel running were seen following this low O_3 concentration. At higher O_3 concentrations (>490 μg/m^3, 0.25 ppm), an increase in the time per wheel revolution, a decrease in the burst length as well as the extended time interval between bursts contributed to the reduced motor activity. These higher concentrations also caused a decrease in performance compared to control for several hours after exposure was terminated.

Effects of O_3 on behavior were further investigated by Weiss et al. (1981) in their studies on the operant behavior of rats during O_3 exposure. The term operant refers to learned behaviors that are controlled by subsequent events such as food or shock delivery. In this case, rats were trained to perform a bar-pressing response maintained by a reward with food pellets delivered according to a 5-min fixed-interval reinforcement schedule. The rats were exposed for 6 hr to O_3 concentrations from 196 to 3920 μg/m^3 (0.10 to 2.0 ppm), with at least 6 days separating successive exposures. Two groups of rats were tested, one beginning in the morning and the other in the mid afternoon. Ozone-induced decreases were linear from 196 to 2744 μg/m^3 (0.10 to 1.40 ppm) for the first group; for the second, the decreases were generally monotonic from 196 to 3920 μg/m^3 (0.10 to 2.0 ppm). Analysis of the distribution of responses during the various O_3 exposures indicated concentration-related decreases arose mainly from the later portions of the sessions and that the onset of the decline in response occurred earlier at the higher O_3 concentrations. In contrast to other types of toxicants, O_3 did not disrupt the temporal pattern that characterized response during each fixed-interval presentation. Based on the sedentary nature of the task, the authors suggested that the inclination to respond rather than the physiological capacity to respond was impaired.

Kulle and Cooper (1975) studied the effects of O_3 on the electrical activity of the nasopalatine nerve in rats. Ozone exposure to 9800 $\mu g/m^3$ (5 ppm) for 1 hr produced an increase in nasopalatine nerve response (action potential frequency) to amyl alcohol, suggesting that the nerve receptors were made more sensitive by prior exposure to O_3. One-hour air perfusion following the O_3 exposure reduced the neural response to amyl alcohol, but not to pre-exposure levels. The nasopalatine nerve is a branch of the trigeminal nerve which responds to airborne chemical irritants. Because most irritants, including O_3, also have odorant properties and, therefore, stimulate both trigeminal and olfactory receptors in the nasal mucosa, it is difficult to distinguish an irritant response from an odor response as the mechanism for behavioral effects in laboratory animals.

Effects of O_3 on the CNS have been reported. Trams et al. (1972) measured biochemical changes in the cerebral cortex of dogs exposed for 18 months to 1960, 3920, or 5880 $\mu g/m^3$ (1, 2, or 3 ppm) of O_3. In 8 hr/day exposures, reported decreases (35 percent) in the catecholamines norepinephrine and epinephrine were not statistically significant, although O_3 exposure at 3920 $\mu g/m^3$ (2 ppm) caused a statistically significant decrease in catechol-o-methyltransferase (COMT) activity. In contrast, monamine oxidase (MAO) activity was significantly elevated at 1960 $\mu g/m^3$ (1 ppm) of O_3, but not at 3920 or 5880 $\mu g/m^3$ (2 or 3 ppm) of O_3. Increasing daily exposures to 1960 $\mu g/m^3$ (1 ppm) from 8 to 24 hr/day caused a significant decrease in COMT activity, but MAO activity increased at 8 and 16 hr/day but decreased at 24 hr/day. Concurrently, Johnson et al. (1976) measured electroencephalographic (EEG) patterns in the same dogs and noted alterations in EEG patterns after 9 months of exposure to 1960 to 5800 $\mu g/m^3$ (1 to 3 ppm) of O_3, but not after 18 months of exposure. The authors noted that it was difficult to correlate the observed EEG changes with the alterations of metabolic balance described. Furthermore, it was even more difficult to assess the metabolic and physiologic significance of the changes without more information about chronic O_3 exposure.

9.4.2 Cardiovascular Effects

Very few reports on the cardiovascular effects of O_3 and other photochemical oxidants in animals have been published. Brinkman et al. (1964) studied structural changes in the cell membranes and nuclei of myocardial muscle fibers in adult mice exposed to O_3. After a 3-week exposure to 392 $\mu g/m^3$ (0.20 ppm) of O_3 for 5 hr/day, structural changes were noted, but these effects were reversible about 1 month after exposure. However, because this study had severe design and methodology limitations, the results should be considered questionable until independently verified.

Bloch et al. (1971) studied the effects of O_3 on pulmonary arterial pressure in dogs. They exposed 31 dogs to 1.0 ppm of O_3 daily for various hours for 17 months. Ten percent (3 dogs) of the animals developed pulmonary arterial hypertension, and approximately 30 percent (9 dogs) had excessive systolic pressure, but there was no proportional relationship between pulmonary arterial hypertension and O_3 exposure. Unless sample sizes were too small to find adequate dose-response effects, the authors attributed the results to genetic susceptibility.

Revis et al. (1981) studied the effects of O_3 and cadmium, singly and combined, in rats. The rats were exposed to 1176 $\mu g/m^3$ (0.60 ppm) O_3, 5 hr/day for 3 consecutive days or to 3 mg/m^3 cadmium for 1 hr or to both pollutants. All exposure treatments resulted in increases in systolic pressure and heart rate. Neither diastolic pressure or mean pressure was affected. No additive or antagonistic effects were seen with the pollutant combinations.

Costa et al. (1983) measured heart rate and standard intervals of cardiac electrical activity from the electrocardiographic (EKG) tracings of rats exposed to 392, 1568, or 3920 $\mu g/m^3$ (0.2, 0.8, or 2 ppm) of O_3 6 hr/day, 5 days/week for 62 exposure days as part of a more extensive evaluation of lung function (Section 9.3.2). Heart rate was not altered by O_3 exposure. The predominant effects occurred at the highest O_3 concentration (3920 $\mu g/m^3$, 2 ppm) at which there was evidence of partial A-V blockade and distorted ventricular activity, often associated with repolarization abnormalities.

Friedman et al. (1983) evaluated the effects of a 4-hr exposure to 588 and 1960 $\mu g/m^3$ (0.3 and 1.0 ppm) of O_3 on the pulmonary gas-exchange region of dogs ventilated through an endotracheal tube. Pulmonary capillary blood flow and arterial O_2 pressure (PaO_2) were decreased 30 min following exposure to both O_3 concentrations, and arterial pH (pH_a) was decreased following exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3. Decreases in pulmonary capillary blood flow persisted 24 hr following exposure to 588 and 1960 $\mu g/m^3$ (0.3 and 1.0 ppm) of O_3 and as long as 48 hr following exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3. Persistent decreases in pH_a and PaO_2 were observed 24 hr following exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3. Pulmonary edema, determined histologically and by increased lung water content and tissue volume, was observed 24 hr following exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3. The data indicate that O_3 exposure can cause both acute and delayed changes in cardiopulmonary function.

9.4.3 Hematological and Serum Chemistry Effects

Hematological effects reported in laboratory animals and man after inhalation of near-ambient O_3 concentrations ($\leq$ 1960 $\mu g/m^3$; $\leq$ 1.0 ppm) indicate that O_3 or some reaction product of O_3 can cross the blood-gas barrier. In addition to reports of morphological and biochemical effects of O_3 on erythrocytes, chemical changes have also been detected in serum after *in vitro* and *in vivo* O_3 exposure. Hematological parameters are frequently used to evaluate O_3 toxicity, because red blood cells (RBCs) are structurally and metabolically simple and well understood, and because the relatively noninvasive methods involved in obtaining blood samples from animals and man make blood samples available for study.

9.4.3.1 Animal Studies - In Vivo Exposures. The effects of *in vivo* O_3 exposure in animals, including studies reviewed in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1978), are summarized in Table 9-18.

Effects of O_3 on the blood were first reported by Christiansen and Giese (1954) after they detected an increased resistance to hemolysis of RBCs from mice exposed to 1960 $\mu g/m^3$ (1.0 ppm) for 30 min. Goldstein et al. (1968) reported a significant decrease in RBC acetylcholinesterase (AChE) activity after exposure of mice to 15,680 $\mu g/m^3$ (8 ppm) of O_3 for 4 hr. Menzel et al. (1975a) observed the presence of Heinz bodies in approximately 50 percent of RBCs in the blood of mice exposed to 1666 $\mu g/m^3$ (0.85 ppm) of O_3 for 4 hr. About 25 percent of RBCs contained Heinz bodies after continuous exposure of mice to 0.85 ppm of O_3 for 3 days. Heinz bodies are polymers of methemoglobin formed by oxidant stress; they appear to attach to the inner membrane of the RBC. However, Chow et al. (1975) detected no significant changes in GSHs, G-6-PDH, oxidized glutathione reductase, or GSH peroxidase in RBCs of rats or monkeys exposed to the same O_3 concentration 8 hr/day for 7 days.

TABLE 9-18. HEMATOLOGY: ANIMAL -- IN VIVO EXPOSURE

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
$\mu g/m^3$	ppm					
110	0.056	c	93 days	Decreased whole blood cholinesterase, which returned to normal 12 days after exposure ceased.	Rat	Eglite, 1968
118 235 470 941	0.6 0.12 0.24 0.48	UV	2.75 hr	RBC survival decreased at 0.06, 0.12, and 0.48 ppm; no concentration-response relationship.	Rabbit	Calabrese et al., 1983a
392	0.2	ND	4 hr	Increased osmotic fragility and spherocytosis of RBC's.	Rabbit	Brinkman et al., 1964
392	0.2	UV	8 hours/day, 5 days/week, 3 weeks	Increased serum glutamic pyruvic transaminase and hepatic ascorbic acid. No change in blood catalase.	Mouse	Veninga, 1970
392	0.2	UV	60 min	Small decrease in total blood serotinin.	Rabbit	Veninga, 1967
392-1960	0.2-1.0	UV	1-4 hr	Plasma creatine phosphokinase activity altered immediately and 15 min postexposure; no effect 30 min postexposure. No change in plasma histamine or plasma lactic acid dehydrogenase.	Mouse	Veninga et al., 1981
490 980 1372	0.25 0.50 0.70	UV	2.75 hr	RBC survival decreased at 0.25 ppm only.	Sheep	Moore et al., 1981a
588	0.3	UV	3 hr/day until death (2-3 wk)	Increased mortality in mice parasitized with Plasmodium berghei. Increased number of parasitized red blood cells.	Mouse	Moore et al., 1984
588	0.3	UV	3 hr	No effect on RBC reduced glutathione, L-ascorbic acid, hemoglobin, red blood cell counts. Slight increase ($p = 0.08$) in % methemoglobin. Decreased hematocrit in O_3-low vitamin C group. Generally, vitamin C deficiency did not increase sensitivity to O_3.	Guinea pig	Ballew et al., 1983

TABLE 9-18. HEMATOLOGY: ANIMAL -- IN VIVO EXPOSURE (continued)

Ozone concentration µg/m^3	Ozone concentration ppm	Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
627	0.32	UV	6 hr ± dietary vitamin E	Increased erythrocyte G-6-PD and decreased AChE (both diets). Increased plasma vitamin E (both diets).	Mouse	Moore et al., 1980
784	0.4	ND	6 hr/day, 5 days/week, 6 months	No change serum trypsin inhibitor capacity.	Rabbit	P'an and Jegier, 1971
784	0.4	ND	6 hr/day, 5 days/week, 10 months	Increase in serum protein esterase.	Rabbit	Jegier, 1973
784	0.4	ND	10 months	Increase in serum protein esterase.	Rabbit	P'an and Jegier, 1972
784	0.4	ND	6 hr/day, 5 days/week, 10 months	Decreased serum albumin concentration. Increased concentration of α- and δ-globulins. Not much change in β-globulin. No change in total serum proteins.	Rabbit	P'an and Jegier, 1976
980	0.5	UV	2.75 hr	Decreased erythrocyte GSH.	Sheep	Moore et al., 1981b
980	0.5	MAST	Continuous, 23 days	Increased hemolysis of erythrocytes of animals depleted of vitamin E. No such change when rats received vitamin E supplements.	Rat	Menzel et al., 1972
980	0.5	NBKI	8 hr/day, 7 days	No change in GSH level or activities of GSH peroxidase, GSH reductase, or G-6-PD in erythrocytes.	Monkey, rat	Chow et al., 1975
1254	0.64	UV	8 hr/day, 1 yr	Altered RBC morphology: decreased number of discocytes, increased number of knizocytes, stomatocytes, and spherocytes. No effect on RBC FA composition.	Monkey	Larkin et al., 1983
1470	0.75	ND	4 hr/day, 4 days	RBC's: Increased fragility; decreased GSH, AChE; no effect on LDH, G-6-PD.	Monkey	Clark et al., 1978

TABLE 9-18. HEMATOLOGY: ANIMAL -- IN VIVO EXPOSURE (continued)

Ozone concentration μg/m^3	 ppm	Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
1568	0.8	NBKI	7 days	Increased activity of GSH peroxidase, pyruvate kinase, and lactate dehydrogenase; and decrease in red cell level of GSH of vitamin E-deficient animals. Animals in both vitamin E-deficient and supplemented diet groups exhibited no change in activities of G-6-DP, catalase, and superoxide dismutase and in levels of thiobarbituric acid reactants, methemoglobin, hemoglobin, and reticulocytes.	Rat	Chow and Kaneko, 1979
1568	0.8	NBKI	8 hr/day, 7 days	No change in total lactate dehydrogenase activity or isoenzyme pattern in plasma or erythrocytes.	Monkey	Chow et al., 1977
1568	0.8	NBKI	Continuous, 29 days	Increased lysozyme activity by day 3.	Rat	Chow et al., 1974
1666	0.85	MAST	4 hr	Increased Heinz bodies in RBC's (decreased with continual exposure).	Mouse	Menzel et al., 1975a
1686	0.86	ND	8 hr/day, 5 days/week, 6 months	Increased infestation and mortality after infection with Plasmodium berghei. Increased acid resistance of erythrocytes.	Mouse	Schlipkoter and Bruch, 1973
1960	1.0	UV	4 hr ± vitamin E	Decreased filterability. No protection by vitamin E. No lipid peroxidation.	Mouse	Dorsey et al., 1983
1960	1.0	ND	30 min	Increased resistance to erythrocyte hemolysis.	Mouse	Mizoguchi et al., 1973; Christiansen and Giese, 1954
1960- 3920	1.0- 2.0	CHEM	2 or 7 days	No changes.	Rat, guinea pig	Cavender et al., 1977

TABLE 9-18. HEMATOLOGY: ANIMAL -- IN VIVO EXPOSURE (continued)

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
μg/m³	ppm					
1960 5880	1.0 3.0	CHEM	4 hr	No effects on oxyhemoglobin affinity, 2,3-DPG concentrations, heme-O_2 binding.	Rabbit	Ross et al., 1979
1960	1	UV	continuously, 2 wk	Increased serum cholesterol, low density lipoproteins and very low density lipoproteins. Males apparently more affected than females. No effect on triglycerides.	Guinea pig	Vaughan et al., 1984
1960 3430 5880	1 1.75 3	CHEM, UV	5 hr/day, 10 days within 14-day period	No effect on serum lipids and lipoproteins at 1 ppm. Concentration related linear increase in total lipoprotein-free cholesterol and high-density lipoprotein total cholesterol; decrease in triglycerides.	Rat	Mole et al., 1985
1960	1	CHEM, UV	5 hr/day, 15 days within 19-day period	Increased serum total cholesterol ($p = 0.1$) high density lipoprotein-cholesterol ($p = 0.08$) and high density lipoprotein-free cholesterol ($p = 0.006$); decrease in triglycerides ($p = 0.06$).		
2940 11,760 15,680	1.5 6.0 8.0	UV	3 days 4 days 4 days	No effect on SOD, GPx, K^+ influx ratios (all levels). Increased Hb, Hct, echinocytes II & III (6 & 8 ppm); echinocytes correlated with petechiae in lungs, indicative of vascular endothelial damage.	Rat	Larkin et al., 1978

[a] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; UV - UV photometry; NBKI = neutral buffered potassium iodide; MAST = KI - coulometric (Mast meter); I = iodometric.

[b] Abbreviations used: RBC = red blood cell; G-6-PD = glucose-6-phosphate dehydrogenase; AChE = acetylcholinesterase; GSH = reduced glutathione; GSH peroxidase = glutathione peroxidase; GSH reductase = glutathione reductase; FA = fatty acid; LDH = lactic dehydrogenase; 2,3-DPG = 2,3-diphosphoglycerate; SOD = superoxide dismutase; GPx = glutathione peroxidase; K = potassium, Hb = hemoglobin; Hct = hematocrit; $PGF_{2}\alpha$ = prostaglandin $F_{2}\alpha$; PGE_2 = prostaglandin E_2.

[c] Spectrophotometric method using dihydroacridine.

In more recent studies, Clark et al. (1978) investigated the biochemical changes in RBCs of squirrel monkeys exposed to 1410 $\mu g/m^3$ (0.75 ppm) of O_3 4 hr/day for 4 days. They observed an increase in RBC fragility with decreases in GSH and AChE activities. No changes were detected in G-6-PDH or lactic dehydrogenase (LDH) activities. After a 4-day recovery period, RBC fragility was still significantly increased, although to a lesser degree. AChE activity returned to control levels at 4 days postexposure; however, RBC GSH remained significantly lowered.

Ross et al. (1979) investigated the effects of O_3 on the oxygen-delivery capacity of erythrocytes. After exposure of rabbits to 1960 or 7880 $\mu g/m^3$ (1 or 3 ppm) of O_3 for 4 hr, no changes were detected in RBC 2,3-diphosphoglycerate concentration, oxyhemoglobin dissociation curve, or heme-oxygen binding of RBCs. Analysis of blood parameters 24 hr after exposure revealed no delayed effects of O_3.

Alterations in RBC morphology have been previously observed in O_3-exposed laboratory animals and man (Brinkman et al., 1964; Larkin et al., 1978). Similar observations have recently been made in monkeys exposed to 1254 $\mu g/m^3$ (0.64 ppm) of O_3 for 8 hr/day over a 1-yr period (Larkin et al., 1983). Ultrastructural SEM studies of RBC's following exposure to O_3 demonstrated reduced numbers of normal discocytes and increased numbers of knizocytes, stomatocytes, and spherocytes, which were either absent or found in small numbers in the blood of air-exposed controls. Despite changes in shape, there were no differences in the fatty acid composition of the erythrocyte total lipids. Values for hematocrit, hemoglobin, mean corpuscular volume, and red cell and reticulocyte count were the same in control and O_3-exposed animals. Moore et al. (1981a) reported reduced RBC survival in sheep exposed to 490 $\mu g/m^3$ (0.25 ppm) of O_3 for 2.75 hr. Similar reductions in RBC survival were reported following 2.75-hr exposures to O_3 concentrations as low as 118 and 235 $\mu g/m^3$ (0.06 and 0.12 ppm) in rabbits (Calabrese et al., 1983a).

Vitamin E deficiency has been associated with an increased hemolysis in rats and other animal species (Scott, 1970; Gross and Melhorn, 1972). Chow and Kaneko (1979) reported significant increases in RBC GSH peroxidase, pyruvate kinase, and LDH activities, and a decrease in RBC GSH after exposure of vitamin E-deficient rats to 1568 ug/m^3 (0.8 ppm) of O_3 continuously for 7 days. These effects were not observed in vitamin E-supplemented rats (45 ppm of vitamin E for 4 months). The activities of G-6-PD, catalase, superoxide dismutase, and levels of TBA reactants, methemoglobin and reticulocytes were not altered by O_3 exposure or by vitamin E status.

Moore et al. (1980) investigated the effects of dietary vitamin E on blood of 9-month-old C57L/J mice exposed to 627 $\mu g/m^3$ (0.32 ppm) of O_3 for 6 hr. Animals were maintained on vitamin E-deficient, or supplemented (3.9 mg tocopherol/100 lb., twice the minimal daily requirement) diets for 6 weeks before O_3 exposure. Mice on the vitamin E-deficient diet showed a 24-percent increase in G-6-PD activity over controls after O_3 exposure, and mice fed a supplemented diet exhibited a 19-percent increase. Decreases in AChE activity were observed in both vitamin E-deficient (19-percent decrease) and vitamin E-supplemented (12-percent decrease) groups.

Dorsey et al. (1983) evaluated the effects of O_3 on RBC deformability after exposure of vitamin E-deficient and supplemented (105 mg of tocopherol per kg of chow) male CD-1 mice to 588 $\mu g/m^3$ (0.3 ppm), 1372 $\mu g/m^3$ (0.7 ppm), or 1960 $\mu g/m^3$ (1.0 ppm) of O_3 for 4 hr. After incubation of RBCs in buffer (0.9 percent RBCs) for up to 6 hr at 25°C, the time required for 2.0 ml of RBC suspension to pass through a 3-µm pore size filter was determined. Exposure of mice to 1960 $\mu g/m^3$ (1.0 ppm) or 1372 $\mu g/m^3$ (0.7 ppm) of O_3 and incubation of RBCs for 6 hr resulted in a significant increase in filtration time of RBCs from O_3-exposed mice, and a lack of protection by dietary vitamin E. The hematocrit of vitamin E-deficient mice exposed to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 was significantly greater than that of nonexposed vitamin E-supplemented mice. The increased hematocrit was attributed to a loss of RBC deformability, and sphering resulting in decreased packing of cells during centrifugation for hematocrit determination. No TBA reactants were detected in the blood of exposed animals, with or without vitamin E.

The influence of vitamin C deficiency on erythrocytes of guinea pigs exposed to 588 $\mu g/m^3$ (0.3 ppm) for 3 hr and examined 0.5 or 3 hr post-exposure was studied by Ballew et al. (1983). Ozone caused no effect on reduced glutathione levels in erythrocytes. There was a slight increase ($p = 0.08$) in percent methemoglobin in O_3-exposed animals. Vitamin C levels did not significantly influence these results. Plasma L-ascorbic acid levels were not affected by O_3 exposure. Hematocrits were decreased in animals on the low vitamin C diet that were exposed to O_3. Hemoglobin and red blood cell counts were unaffected by O_3.

Moore et al. (1984) infected mice with Plasmodium berghei (a blood-borne malarial parasite) 1 day prior to exposure to 588 $\mu g/m^3$ (0.3 ppm) O_3. The exposure lasted for 3 hr/day until death, or approximately 2 to 3 wk. Mice exposed to O_3 did not live as long as the controls. Ozone-exposed mice also had an increase in the number of parasitized red blood cells. The authors hypothesize that there are 2 potential mechanisms responsible: O_3 may have altered the erythrocyte membrane, making it more permeable to P. berghei, or O_3 increased the reticulocyte count, reticulocytes possibly being more sensitive to P. berghei infestation. These results are consistent with those of Schlipköter and Bruch (1973), who reported, without statistical analysis, an increase in infestation with P. berghei and higher mortality in mice exposed for 6 mo (8 hr/day, 5 days/wk) to 1686 $\mu g/m^3$ (0.86 ppm) O_3.

9.4.3.2 In Vitro Studies. The effects of in vitro O_3 exposure of animal blood have been studied by a number of investigators, and these reports are summarized in Table 9-19.

The effects of in vitro O_3 exposure on human RBCs have been evaluated by using a number of different end points, such as increases in complement-mediated cell damage (Goldstein et al., 1974a), formation of Heinz bodies (Menzel et al., 1975b), decreases in RBC native protein fluorescence (Goldstein and McDonagh, 1975), and decreases in concanavalin A agglutinability (Hamburger et al., 1979). Exposure of RBCs or their membranes to O_3 has also been shown to inhibit (Na+ - K+) ATPase (Kindya and Chan, 1976; Chan et al., 1977; Koontz and Heath, 1979; Freeman et al., 1979; Freeman and Mudd, 1981). Kindya and Chan (1976) proposed that inhibition of ATPase by O_3 caused spherocytosis and increased fragility of RBCs after O_3 exposure. (See Table 9-20 for a summary of the human in vitro studies.)

Kesner et al. (1979) demonstrated that O_3-treated phospholipids inhibited RBC membrane ATPase. Addition of semicarbazide to O_3-exposed phospholipids before mixing with RBC membranes substantially reduced the inhibitory effect, suggesting that the inhibitors may be carbonyl compounds. In addition, a slower-forming semicarbazide-insensitive inhibitor was formed.

Verweij and Steveninck (1980, 1981) reported that semicarbazide and also p-aminobenzoic acid (PABA) might protect by acting as O_3 scavengers. Spectrin (a major glycoprotein component of the RBC membrane) solution was treated by bubbling O_3-containing O_2 through the solution at 4 ml/min (2.5 μM/min of O_3) for 1 or 2 min. Semicarbazide (40 μM) or PABA (40 μM) inhibited the cross-linking of O_3-exposed spectrin. The inhibition of AChE and hexokinase activities of RBC ghosts exposed to O_3 was also partially prevented by these two agents, as was K^+ influx into whole RBCs. The authors attributed the inhibition

TABLE 9-19. HEMATOLOGY: ANIMAL -- IN VITRO EXPOSURE

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
980- 3920	0.5 2.0	CHEM	2 hr	Decrease in agglutination of erythrocytes by concanavalin A.	Rat	Hamburger and Goldstein, 1979
1960- 13,132	1.0 6.7	NBKI	90 min- 4 hr	Decreased erythrocyte catalase levels at $\geq$ 5 ppm when animals were pretreated with aminotriazole.	Rat, mouse	Goldstein, 1973
2156 4508	1.1 2.3	UV	16 hr	No effect on hemoglobin. No change in organic free radicals as measured by EPR spectra. No statistics.	Mouse	Case et al., 1979

[a] Measurement method: CHEM = gas phase chemiluminescence; UV = UV photometry; NBKI = neutral buffered potassium iodide.

TABLE 9-20. HEMATOLOGY: HUMAN - IN VITRO EXPOSURE

Ozone[a] concentration	Measurement[b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
980 µg/m^3 (0.5 ppm) 1960 µg/m^3 (1.0 ppm)	CHEM	0.5-2 hr	Decreased agglutination of RBCs by concanavalin A.	Human	Hamburger et al., 1979
O_3-treated phospholipids	ND	5, 10, 15, and 20 min	Decreased ATPase activity.	Human (RBC ghosts)	Kesner et al., 1979
4 µM/min	I	1 min	Decreased ATPase activity.	Human (RBC ghosts)	Kindya and Chan, 1976
Methylozonide 10^{-4}-2×10^{-3} M	ND	30 min	Heinz body formation. Prevented by dietary vitamin E.	Human	Menzel et al., 1975b
750 nM/min	NBKI	14.3 or 43.0 nMol of O_3 per 10^6 cell equivalent	RBC -- No effect on ATPase. Decreased cation transport. RBC ghosts -- decreased ATPase activity.	Human	Koontz and Heath, 1979
106 nM/min	NBKI	5, 10, 20, 30, 40 and 50 min	Decreased activity of purified α_1-proteinase inhibitor.	Human	Johnson, 1980
300 nM/min	NBKI	ND	Decreased glyceraldehyde-3-PD. Decreased ATPase. No statistics.	Human (RBC ghosts)	Freeman et al., 1979
0-9.8 µM/g of Hb 0.84 µM/min	NBKI	0-2 hr	Decreased GSH. No effect on Hb or on glucose uptake.	Human (RBCs, RBC ghosts)	Freeman and Mudd, 1981
78400 µg/m^3 (40 ppm)	NBKI	2 hr	Increased complement-mediated cell damage.	Human	Goldstein et al., 1974a
1,960 µg/m^3 (1.0 ppm)	NBKI	20 and 60 min	Decreased native protein fluorescence. No statistics.	Human (RBC ghosts)	Goldstein et al., 1975

TABLE 9-20. HEMATOLOGY: HUMAN - IN VITRO EXPOSURE (continued)

Ozone[a] concentration	Measurement[b] method	Exposure duration and protocol	Observed effect(s)	Species	Reference
40 nM/min	I	4 min	Decreased ATPase activity; lost 40% membrane sulfhydryls. Lipid peroxidation and protein crosslinking detected. Pretreatment with semicarbazide prevented crosslinking.	Human (RBC ghosts)	Chan et al., 1977
2.5 μM/min	I	20, 40, and 60 min	Cross-linking of membrane proteins inactivation of glyceraldehyde-3-phosphate dehydrogenase.	Human	Verweij and Steveninck, 1980
2.5 μM/min	I	20, 40, and 60 min	Crosslinking of spectrin. Decreased AChase activity. Increased K+ leakage from RBCs. Semicarbazide and p-amino benzoic acid prevented these O_3 effects.	Human (RBC ghosts)	Verweij and Van Steveninck, 1981

[a] Not ranked by concentration; listed by reported values.

[b] Measurement method: ND = not described; CHEM = gas phase chemiluminescence; NBKI = neutral buffered potassium iodide; I = iodometric.

of ATPase to oxidation of phospholipids with subsequent cross-linking of membrane protein by lipid peroxidation products. Because the reaction of ozonolysis products with semicarbazide and PABA during O_3 treatment of RBCs was not directly measured in these studies, the protective mechanism remains unclear.

In a recent study, Freeman and Mudd (1981) investigated the *in vitro* reaction of O_3 with sulfhydryl groups of human RBC membrane, proteins, and cytoplasmic contents. After exposure of RBCs to O_3 in O_2 at 20 ml/min (0.84 μMol/min of O_3) for up to 2 hr, oxidation of intracellular GSH was observed. Ozone exposure produced membrane disulfide cross-links in RBC ghosts but not in intact RBCs. Neither oxyhemoglobin content nor glucose uptake was affected by O_3 exposure of RBCs. These data support earlier studies of Menzel et al. (1972) that reported decreased RBC GSH levels following exposure of rats to 980 $\mu g/m^3$ (0.5 ppm) of O_3 continuously for 23 days.

Although *in vitro* studies using animal and human RBCs have provided information on the possible mechanism by which O_3 may react with cell membranes and RBCs, extrapolation of these data to *in vivo* O_3 toxicity in man is difficult. In most *in vitro* studies, RBCs were exposed by bubbling high O_3 concentrations (> 1 ppm) through cell suspensions. Not only were the O_3 concentrations unrealistic and the method of exposure nonphysiological, but the toxic species causing RBC injury may be different during *in vitro* and *in vivo* O_3 exposures. Because of its reactivity, it is uncertain that O_3 *per se* reaches the RBCs after inhalation but may instead appear in blood in the form of less reactive products (e.g., lipid, peroxides). However, during *in vitro* exposure of RBC suspensions, O_3 or highly reactive free-radical products (e.g., hydroxyl radical, superoxide anion, singlet oxygen) may be the cause of injury.

9.4.3.3 *Changes in Serum*. In addition to O_3's effects on RBCs, changes have been detected in the serum of animals exposed to O_3. P'an and Jegier (1971) investigated the effects of 784 $\mu g/m^3$ (0.4 ppm) of O_3 6 hr/day, 5 days/week for 6 months on the serum trypsin inhibitor capacity (TIC) of rabbits. With the exception of a sharp rise after the first day of exposure, TIC values remained within normal limits. However, after exposure for 10 months, the TIC had progressively increased to about three times the normal level (P'an and Jegier, 1972). Microscopic evaluation suggested that the rise in TIC may have been due to the thickening of small pulmonary arteries. The results from this study are questionable, however, because the rabbits may have had intercurrent infectious disease, which was more severe in the exposed animals (Section 9.3.1).

P'an and Jegier (1976) also reported changes in serum proteins after exposure of rabbits to 784 μg/m^3 (0.4 ppm) and 1960 μg/m^3 (1.0 ppm) of O_3. Following exposure to 784 μg/m^3 (0.4 ppm) of O_3 for 105 days, the albumin concentrations began to decrease, and α- and δ-globulin concentrations began to increase. At the end of 210 days of exposure, the mean albumin level fell 16 percent, the α-globulin level rose 78 percent, and the δ-globulin levels fell 46 percent. No significant changes were observed in total protein concentration.

Chow et al. (1974) observed that the serum lysozyme activity of rats increased significantly during continuous (24 hr/day) but not during intermittent (8 hr/day) exposure to 1568 μg/m^3 (0.8 ppm) of O_3 for 7 days. The increased release of lysozyme into the plasma was suggested to be a result of O_3 damage to alveolar macrophages.

Veninga et al. (1981) reported that short-term exposures of mice to low O_3 concentrations induced changes in serum creatine phosphokinase (CPK) activity. Ozone doses were expressed as the product of concentration and time; the maximum O_3 concentration was 1600 μg/m^3 (0.8 ppm), and the maximum exposure time was 4 hr. Alterations in CPK were detected immediately and 15 min after termination of the exposure. By 30 min postexposure, the CPK activities had returned to control levels. Neither plasma histamine nor plasma LDH was altered by the range of O_3 doses employed. The authors concluded that these responses may represent adaptation of the animals to O_3 toxicity by enhanced metabolic processes.

Serum lipids and lipoproteins of rats exposed continuously for 2 wk to 1960 μg/m^3 (1 ppm) were determined (Vaughan et al., 1984). Serum from each guinea pig was sampled before and immediately after the 2-wk exposure and 30 days after exposure ceased. Thus, each animal served as its own control and there was no air-exposure group. Immediately after exposure, cholesterol, low density lipoproteins, and very low density lipoproteins were elevated in males. Generally, females had similar effects, but no changes in very low density lipoproteins. Triglycerides were not affected in either sex immediately after exposure. Although statistical comparisons of sex susceptibility were not performed, it appears that males were more affected than females. Statistical tests of the post-exposure group were unclear, but it appears that levels of cholesterol, low density lipoproteins, and very low density lipoproteins had returned to pre-exposure values.

Serum lipids and lipoproteins have also been evaluated in male rats after repeated O_3 exposure (Mole et al., 1985). A concentration response study involved exposure to air; 1960, 3430, and 5880 $\mu g/m^3$ (1, 1.75, and 3 ppm) O_3 for 5 hr/day for 10 exposure days within a 14-day period. For a given rat, serum samples taken 2 days prior to the first exposure were compared to samples taken 20 hr after the last exposure; each animal served as its own control. Another group of animals was exposed to air and sampled pre- and postexposure. Shifts in the O_3-exposed rats (pre- vs. postexposure) were compared statistically to shifts in the air-exposed animals. Ozone caused a concentration-related linear increase in total lipoprotein-free cholesterol and high density lipoprotein total cholesterol (both the free and esterified components) and a decrease in total lipoprotein triglycerides. There was no effect on high density lipoprotein-tryglycerides or on total lipoprotein-free fatty acids. At the 1960 $\mu g/m^3$ (1 ppm) O_3 level, none of the values was elevated significantly over controls. In a sampling time study (Mole et al., 1985), rats were exposed to 1960 $\mu g/m^3$ (1 ppm) O_3 for 5 hr/day for 15 days in 5-day segments within a 19-day period. Serum samples were taken from each rat 4 days prior to the first exposure and at 7 times (0 to 44 hr) after the last exposure. Ozone increased serum total chlolesterol ($p = 0.1$), high density lipoprotein-cholesterol ($p = 0.08$), and high density lipoprotein-free cholesterol ($p = 0.006$), and decreased triglycerides ($p = 0.06$). The changes appeared to be maintained over the 44-hr post-exposure period and were greater than those observed at the 1960 $\mu g/m^3$ (1 ppm) O_3 level of the concentration-response study described above that used fewer days of exposure. Thus, O_3 caused a mild hypercholesterolemia and hypotriglyceridemia.

Both the Vaughan et al. (1984) and Mole et al. (1985) studies report increases in serum cholesterol. There is some disparity between results for other lipoproteins. The Vaughan et al. study did not account for changes that can be produced by exposure stress alone, as indicated by Mole et al. (1985). The Mole et al. (1985) studies showed only marginally significant effects. Thus, a possible conclusion from the rat and guinea pig studies is that short-term exposure to 1960 $\mu g/m^3$ (1 ppm) has the potential of elevating cholesterol in animals. Elevation in human serum cholesterol is a risk factor in human coronary heart disease (Gotto, 1979; Dawber, 1980).

9.4.3.4 Interspecies Variations. The use of animal models to investigate the effects of O_3 on the blood is complicated, because few species respond like humans. The rodent model has been most commonly used to predict the effects of O_3 on human RBCs (Calabrese et al., 1979). However, the reliability of this model was challenged by Calabrese and Moore (1980) on the following grounds: (1) ascorbic acid synthesis was significantly increased in mice following O_3 exposure (Veninga and Lemstra, 1975), (2) ascorbic acid protected human G-6-PD-deficient RBCs *in vitro* from the oxidant stress of acetylphenylhydrazine (Winterbourn, 1979), and (3) humans lack the ability to synthesize ascorbic acid. Although Calabrese and Moore (1980) stressed that this hypothesis is based on a very limited data base, they point out the importance of developing animal models that can accurately predict the response of human G-6-PD-deficient humans to oxidant stressor agents. In another report, Moore et al. (1980) suggested that C57L/J mice may present an acceptable animal model, because these mice responded to O_3 exposure (627 μg/m^3, 0.32 ppm for 6 hr) in a manner similar to that of humans, with increases in serum vitamin E and G-6-PD activity. Unlike many other mouse strains, the C57L/J strain has low G-6-PD activity, which is similar to that found in human RBCs. Moore et al. (1981b) also followed up on the proposed use of Dorset sheep as an animal model for RBC G-6-PD deficiency in humans (National Research Council, 1977). However, Dorset sheep were found to be no more sensitive than normal humans with respect to O_3-induced changes in GSH and also differed from humans in the formation of methemoglobin. Further studies (Calabrese et al., 1982, 1983b,d; Williams et al., 1983a,b,c) demonstrated that the responses of sheep and normal human erythrocytes were very similar when separately incubated with potentially toxic O_3 intermediates, but G-6-PD-deficient human erythrocytes were considerably more susceptible. Consequently, the authors also questioned the value of the sheep erythrocyte as a quantitatively accurate predictive model.

9.4.4 Reproductive and Teratogenic Effects

Pregnant animals and developing fetuses may be at greater risk to effects from photochemical oxidants, because the volume of air inspired by females generally increases from 15 to 50 percent during pregnancy (Altman and Dittmer, 1971). Before 1978, experiments designed to investigate the reproductive effects of photochemical oxidants often used complex mixtures of gases, such as irradiated auto exhaust (see Section 9.5), or they used oxidant concentrations greater than those typically found in ambient air. Brinkman et al.

(1964) exposed pregnant mice to lower concentrations of O_3, but the results of their experiments are difficult to interpret, because the time of O_3 exposure during gestation and postparturition was not specified. They reported that mice exposed to 196 or 392 $\mu g/m^3$ (0.1 or 0.2 ppm) of O_3 for 7 hr/day and 5 days/week over 3 weeks had normal litter sizes, compared with air-exposed controls. However, there was greater neonatal mortality in the litters of O_3-exposed mice, even at the exposure level of 196 $\mu g/m^3$ (0.1 ppm) of O_3 (Table 9-21). Unfortunately, without more details on the period of exposure, it is impossible to ascertain whether the decreased infant survival rate was due to development interference *in utero*, to a direct effect on the pups, or to a nutritional deficiency caused by parental anorexia or reduced lactation, or a combination of these effects. When using a similar experimental protocol, Veninga (1967) found that mice exposed to 392 $\mu g/m^3$ (0.2 ppm) of O_3 for 7 hr/day, 5 days/week during embryological development and the 3 weeks after birth (total exposure time not reported) had an increased incidence of excessive tooth growth, although no statistical evaluation was provided.

In more recent experiments, Kavlock et al. (1979) exposed pregnant rats to O_3 for precise periods during organogenesis. No significant teratogenic effects were found in rats exposed 8 hr/day to concentrations of O_3 varying from 863 to 3861 $\mu g/m^3$ (0.44 to 1.97 ppm) during early (days 6 to 9), mid (days 9 to 12), or late (days 17 to 20) gestation, or the entire period of organogenesis (Days 6 to 15). Continuous exposure of pregnant rats to 2920 $\mu g/m^3$ (1.49 ppm) of O_3 in midgestation resulted in increased resorption of embryos. A single dose of 150 mg/kg sodium salicylate followed by 1960 $\mu g/m^3$ (1.0 ppm) of O_3 during midterm produced a significant synergistic increase in the resorption rate, a decrease in maternal weight change, and a decrease in average fetal weight. Exposure of pregnant rats 8 hr/day to 862 $\mu g/m^3$ (0.44 ppm) of O_3 throughout the period of organogenesis also resulted in a significant decrease in average maternal weight gain.

In a follow-up study, Kavlock et al. (1980) investigated whether *in utero* exposure to O_3 can affect postnatal growth or behavioral development. In contrast to the results of Brinkman et al. (1964), neonatal mortality of rats was not increased by exposure to 2940 $\mu g/m^3$ (1.5 ppm) of O_3 for periods of 4 days during gestation. Pups from litters of females exposed to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 during mid- (days 9 to 12) or late (days 17 to 20) gestation exhibited significant dose-related reductions in weight 6 days after birth. Pups

TABLE 9-21. REPRODUCTIVE AND TERATOGENIC EFFECTS OF OZONE

Ozone concentration $\mu g/m^3$	ppm	Measurement method[a]	Exposure duration and protocol	Observed effect(s)	Species	Reference
196	0.1	ND	7 hr/day, 5 days/week for 3 weeks	Increased neonatal mortality (4.9 to 6.8% vs. 1.6 to 1.9% for controls)[b].	Mouse	Brinkman et al., 1964
392	0.2	ND	7 hr/day, 5 days/week for 3 weeks	Unlimited growth of incisors (5.4% incidence vs. 0.9% in controls)[b].	Mouse	Veninga, 1967
862	0.44	I	8 hr/day over entire period of organogenesis (days 6 to 15)	Decreased average maternal weight gain.	Rat	Kavlock et al., 1979
2920	1.49		Continuous during mid-gestation	Increased fetal resorption rate (50% vs. 9% for controls).		
1960	1.0	I	Continuous during late gestation	Slower development of righting, eye opening, and horizontal movement; delayed grooming and rearing behavior.	Rat	Kavlock et al., 1980
1960	1.0		Continuous during mid- (day 9 to 12) or late (days 17 to 20) gestation	Average weight reduced 6 days after birth.		
2940	1.5		Continuous during late gestation (days 17 to 20)	3 males (14.3%) were permanently runted.		

[a] Measurement method: ND = not described, I = iodometric (Saltzman and Gilbert, 1959).

[b] No statistical evaluation.

from the late gestation exposure group were affected to a greater extent and for a longer period of time after parturition. In fact, several males exposed to 2940 μg/m^3 (1.5 ppm) of O_3 during late gestation were also significantly slower in the development of early movement reflexes and in the onset of grooming and rearing behaviors. The authors pointed out that it is impossible to distinguish between prenatal and postnatal contributions to the behavioral effects, because foster parent procedures were not used to raise the pups.

9.4.5 Chromosomal and Mutational Effects

9.4.5.1 Chromosomal Effects of Ozone. A large portion of the data available on the chromosomal and mutational effects of O_3 was derived from investigations conducted above 1,960 μg/m^3 (1 ppm) of O_3, and their relevance to human health is questionable. However, for completeness of the review of the literature, and for possible insight into the mechanisms by which O_3 may produce genotoxicity, this discussion will not be limited to data derived from research conducted at or below 1,960 μg/m^3 (1 ppm) of O_3. Data derived predominantly from *in vitro* experiments conducted at O_3 concentrations in excess of 1,960 μg/m^3 (1 ppm) of O_3 will be discussed first (Table 9-22), followed by a discussion of the genotoxicity data from both *in vitro* and *in vivo* research conducted at or below 1 ppm of O_3 (Table 9-23).

The potential for genotoxic effects relating to O_3 exposure was predicted from the radiomimetic properties of O_3. The decomposition of O_3 in water produces OH and HO_2 radicals, the same species that are generally considered to be the biologically active products of ionizing radiation. Fetner (1962) reported that chromatid deletions were induced in a time-dependent manner in human KB cells exposed to 15,680 μg/m^3 (8 ppm) of O_3 for 5 to 25 min. The chromatid breaks were apparently identical to those produced by x-rays. A 10-min exposure to 8 ppm of O_3 was slightly more efficient in the production of chromatid breaks than 50 rad of x-rays. Significant mitotic delay was measured in neuroblasts from grasshoppers (*Chortophaga viridifaciata*) exposed to 3500 to 4500 μg/L of O_3 in a closed system (Fetner, 1963).

Scott and Lesher (1963) measured a sharp loss of viability with *Escherichia coli* as the O_3 concentration was increased. Viability was reduced to zero when cells were exposed to 1 μg/ml of O_3. Damage to cell membranes was evident by the leakage of nucleic acids and other cellular components from cells exposed to 0.18 μg/ml of O_3.

TABLE 9-22. CHROMOSOMAL EFFECTS FROM *IN VITRO* EXPOSURE TO HIGH OZONE CONCENTRATIONS

O_3 concentration[a]	Measurement method[b]	Exposure duration and protocol[c]	Observed effect(s)	Species	Reference
15,680 μg/m³ (8ppm)	UKI	5-25 min	Chromatid deletions.	Humans KB cells	Fetner, 1962
98,000 μg/m³ (50 ppm)	MAST[d]	30 min	*lex* mutants deficient in repair of x-ray-induced DNA strand breaks were more sensitive to lethal effects of O_3 than were the wild-type repair-proficient parental strains	*Escherichia coli*	Hamelin and Chung, 1974
98,000 μg/m³ (50 ppm)	MAST[d]	30 min	DNA Polymerase I mutant strains (KMBL 1787, 1789, 1791) were more sensitive to the cytotoxic effects of O_3, and DNA was degraded to a greater extent in the first 3 hr after O_3 exposure than strain KMBL 1788, which contains a normal DNA Polymerase I.	*E. coli*	Hamelin et al., 1977a
98,000 μg/m³ (50 ppm)	MAST[d]	30 min	Mucoid mutant strains (MQ 100 & 105) obtained by treating MQ 259 with O_3 yet having full complement of DNA repair enzymes were shown to be more sensitive to O_3 and degraded DNA to a greater extent than the *lon* + (MQ259) strain.	*E. coli*	Hamelin et al., 1977b
98,000 μg/m³ (50 ppm)	MAST[d]	up to 3 hrs	15 different DNA repair-deficient strains were tested for sensitivity to the cytotoxic effects of O_3; DNA Polymerase I was involved in DNA repair but Polymerases I and II and DNA synthetic genes dna A, B, and C were not; recombinational repair pathways, assayed with rec A and rec B strains, were only partially involved in the repair of O_3-induced DNA damage.	*E. coli*	Hamelin and Chung, 1978

TABLE 9-22. CHROMOSOMAL EFFECTS FROM *IN VITRO* EXPOSURE TO HIGH OZONE CONCENTRATIONS (continued)

O_3 concentration[a]	Measurement method[b]	Exposure duration and protocol[c]	Observed effect(s)	Species	Reference
0.1 μg/ml [51 ppm]	UV	60 min (70 ml/min)	Preferential degradation of yeast RNA at the N-glycosyl linkage; sugar-phosphate linkage was O_3 stable.	Yeast	Shiniki et al., 1983
0.5 μg/ml [255 ppm]	UV	30 min (330 ml/min)	5-ribonucleotide guanosine monophosphate was degraded most rapidly.		
0.18 μg/ml [92 ppm] 1.0 μg/ml [510 ppm]	UKI	ND	Release of nucleic acids; cell lethality.	*E. coli*	Scott and Lesher, 1963
0.5-6 μg/ml [255-3061 ppm]	NBKI	0-5 min	O_3 reacts with pyrimidine bases from nucleic acids (thymidine > cytosine > uracil).	*E. coli*	Prat et al., 1968
1-10 μg/ml [510-5100 ppm]	NBKI	30 min	Cell death and nonspecific chromosomal aberrations: shrunken and fragmented nuclei, clumped metaphase chromosomes and chromosome bridges.	Chick embryo fibroblasts	Sachsenmaier et al., 1965
5% [50,000 ppm]	NBKI	0-40 min	Rapid loss of glycolytic and respiratory capacity; loss of tumorigenicity after 20 min. exposure.	Mouse ascites cells	
3.5-4.5 μg/ml [1786-2296 ppm]	UKI	ND	Mitotic delay.	*Chortophaga viridifaciata*	Fetner, 1963
2% [20,000 ppm]	e	3 min	Abnormal nuclei; fragmentation.	Am. oyster	MacLean et al., 1973
8% [80,000 ppm]	GPT	5, 15, 60 s	Rapid degradation of nucleic acid bases, nucleosides or nucleotides in 0.05M phosphate buffer, pH 7.2.	ND	Christensen and Giese, 1954

[a] Not ranked by air concentration; listed by reported exposure values and [approximate ppm conversions].

[b] Measurement method: MAST = KI-coulometric (Mast meter); NBKI = neutral buffered potassium iodide; UV = UV photometry; GPT = gas phase titration; UKI = unbuffered potassium iodide.

[c] O_3 flow rate given in (ml/min), when available. ND = not described.

[d] Concentrations of O_3 were not measured in the cell suspensions.

[e] O_3 analyzer (Fisher and Porter, Warminster, PA).

TABLE 9-23. CHROMOSOMAL EFFECTS FROM OZONE CONCENTRATIONS AT OR BELOW 1960 µg/m³ (1 ppm)

O_3 Concentration		Measurement[a,b] method	Exposure[c] duration and protocol	Observed effect(s)[d]	Species	Reference
µg/m³	ppm					
294	0.15	NBKI	5 hr	No effect induced by O_3 treatment on the frequency of chromosome or chromatid aberrations in Chinese hamster or mouse peripheral blood lymphocytes stimulated with PHA; no effect on spermatocytes in mice 8 wk following exposure.	Mouse	Gooch et al., 1976
412	0.21		5 hr			
1940	0.99		2 hr			
451	0.23	NBKI	5 hr (*in vitro*)		Hamster	
2548-14,700	1.3-7.5	NBKI	ND (*in vitro*)	Peripheral blood lymphocytes exposed to O_3 in culture 12 hr after stimulation with PHA showed no increase in chromosome or chromatid aberrations.	Human lymphocytes	
3234-27,832	1.65-14.2	NBKI	ND (*in vitro*)	Peripheral blood lymphocytes exposed to O_3 in culture, 36 hr after PHA stimulation, showed no change in the frequency of chromosome or chromatid aberrations at any concentration except 7.23 ppm of O_3.	Human lymphocytes	
3920	2.0	NBKI	5-90 min (*in vitro*)	No apparent increase in the frequency of chromosome or chromatid aberrations 12 or 36 hr after PHA stimulation.	Human lymphocytes	
392	0.2	MAST UKI	5 hr (*in vivo*)	Combined exposure to O_3 and radiation (227-233 rad) produced an additive effect on the number of chromosome breaks measured in peripheral blood lymphocytes.	Hamster	Zelac et al., 1971b
470-588	0.24-0.3	MAST UKI	5 hr (*in vivo*)	A significant increase in chromosome aberrations (deletions, ring dicentrics) in the peripheral blood lymphocytes; increased break frequency was still apparent at 6 and 16 days following exposure.	Hamster	Zelac et al., 1971a

TABLE 9-23. CHROMOSOMAL EFFECTS FROM OZONE CONCENTRATIONS AT OR BELOW 1960 μg/m³ (1 ppm) (continued)

O_3 Concentration μg/m³	O_3 Concentration ppm	Measurement[a,b] method	Exposure duration and protocol[c]	Observed effect(s)[d]	Species	Reference
490-1960	0.25-1.0	UV	1 hr (*in vitro*)	Dose-related increase in SCE frequency in WI-38 diploid fibroblasts exposed in culture.	Human fibroblasts	Guerrero et al., 1979
588-1568	0.3-0.8	UV	8 days, continuous (*in vitro*)	Growth of cells from lung, breast, and uterine tumors were inhibited to a greater degree than IMR-90, a nontumor diploid fibroblast.	Human tumor cells	Sweet et al., 1980
843	0.43	UV, NBKI	5 hr (*in vivo*)	Increase in chromatid-type aberrations in peripheral blood lymphocytes of O_3-exposed hamsters; Increase in deletions at 7 days and increase in achromatic lesions at 14 days after exposure; chromosome-type lesions were not significantly different; no chromosomal aberrations in bone marrow lymphocytes; no change in SCE frequency in peripheral blood lymphocytes.	Hamster	Tice et al., 1978
3920	2.0		6 hr (*in vivo*)	No change in SCE frequency in peripheral blood lymphocytes.	Mouse	
1960-9800	1.0-5.0	CHEM NBKI	24 hr (*in vivo*)	Variable decrease in the molecular weight of DNA from peritoneal exudate cells of O_3 exposed mice becoming significant at 5 ppm; significant induction of single-strand breaks at 5 ppm.	Mouse	Chaney, 1981

[a] Measurement method: MAST = KI-coulometric (Mast meter); NBKI = neutral buffered potassium iodide; UV = UV photometry

[b] Calibration method: UKI = unbuffered potassium iodide; NBKI = neutral buffered potassium iodide.

[c] ND = not described.

[d] Abbreviations used: PHA = phytohemagglutinin; SCE = sister chromatid exchange.

The molecular mechanism for the clastogenic and lethal effects resulting from O_3 exposure are not precisely known. Bubbling 8 percent O_3 through a phosphate buffer solution (0.05M, pH 7.2) containing DNA caused an immediate loss in absorption at 260 nm and an increase in the absorption of the solution at wavelengths shorter than 240 nm (Christensen and Giese, 1954). A similar rate of degradation was observed with RNA and the individual purine and pyrimidine bases, nucleosides, and nucleotides. In a more recent report, Shiniki et al. (1983) examined the degradation of a mixture of 5' nucleotides, yeast t-RNA or tobacco mosaic virus RNA with O_3 (0.1 to 0.5 mg/L). The guanine moiety was found to be the most O_3-labile among the four nucleotides, whether the guanine was present as free guanosine monophosphate or incorporated into RNA. The sensitivity to degradation by O_3 among the four nucleotides was found to be, in decreasing order, GMP > UMP > CMP > AMP (GMP = guanosine monophosphate, U = uridine, C = cytidine, A = adenosine). Even after extensive ozonolysis of yeast t-RNA (0.5 μg/ml, 30 min) and substantial degradation of the guanine moieties, the RNA migrated as a single band on polyacrylamide gels. The band exhibited the same mobility as the intact t-RNA, indicating that although the glycosidic bond between the sugar and the base is O_3-labile, the sugar-phosphate backbone was intact and extremely stable against O_3. Prat et al. (1968) investigated the reactivity of the pyrimidines in E. coli DNA with O_3 (0.5 to 6 mg/L, 0 to 5 min) and radiation. Ozone preferentially reacted with thymidine, then with cytosine and uracil, in decreasing order of reactivity. The results are slightly different from those reported by Shiniki et al. (1983) in that the reactivity with uracil and cytidine are in reversed order.

There is evidence that single-strand breaks in DNA may contribute to the genotoxic effects of O_3. Radiosensitive lex mutants of E. coli, which were known to be defective in the repair of x-ray-induced single-strand breaks in DNA, were found to be significantly more sensitive to the cytotoxic effects of O_3 than the repair-proficient parental strain (Hamelin and Chung, 1974).

In an effort to investigate the nature of the O_3-induced lesion in DNA, Hamelin and co-workers investigated the survival of bacterial strains with known defects in DNA repair. Closely related strains of E. coli K-12 with mutations in DNA polymerase I were shown to be more sensitive to the cytotoxic effects of O_3 than the DNA polymerase proficient (pol +) strain (Hamelin et al., 1977a). Polymerase I-deficient strains also exhibited an extensive degradation of DNA in response to O_3 or x-ray treatment. The authors concluded that DNA polymerase I plays a key role in the repair of lesions produced in E. coli DNA by O_3 and that the unrepaired damage was responsible for the enhanced degradation of DNA and the enhanced cell killing observed in the pol- mutants. This interpretation of the data may not be entirely correct, because an enhanced degradation of DNA and an increased sensitivity to cell killing were also observed in a lon mutant strain of E. coli K-12 (Hamelin et al., 1977b). The lon mutant appears to have a full complement of DNA repair enzymes. With these mutants, there may be an enhanced DNA repair activity (evidenced by the extensive degradation of DNA), and the enhanced activity of the lon gene products was thought to be responsible for the increased cell killing observed with these strains when they were exposed to O_3.

Although DNA polymerase I was shown to be involved in the repair of O_3-induced DNA damage (Hamelin et al., 1977a), E. coli cell strains with mutations in DNA polymerase II or III were not found to be more sensitive to O_3 than the wild-type, suggesting that these enzymes are not involved in the repair of DNA damaged by O_3 (Hamelin and Chung, 1978). Mutant strains of E. coli with defects in DNA synthesis (DNA A, B, C, D, and G) showed no enhanced sensitivity to O_3. Therefore, the DNA gene products are probably not involved in the repair of O_3 damage. Recombinational repair mutants, rec A and rec B, only showed a slightly increased sensitivity to O_3 than the wild-type, suggesting that the rec gene products are only partially involved in the repair of O_3-induced DNA lesions (Hamelin and Chung, 1978).

Other effects have been observed than those described above on bacteria. In the commercial American oyster exposed to O_3-treated sea water (MacLean et al., 1973), fertilization occurred less readily and abnormal nuclei (degeneration, fragmentation) were observed approximately twice as frequently. Sachsenmaier et al. (1965) observed a rapid loss of glycolytic and respiratory capacity and subsequent loss of tumorigenicity in mouse ascites cells treated with O_3. These authors also reported that chicken embryo fibroblasts exposed to O_3 (1 to 10 μl/ml) for 30 min exhibited nonspecific alterations in cells resembling those seen after x-ray damage, including shrunken nuclei, clumped metaphase chromosomes, arrested mitosis, chromosome bridges and fragmented nuclei.

In the studies described up to this point, the investigators have predominantly examined the in vitro effects of extremely high O_3 concentrations on biological systems or biologically important cellular components. Although these investigations may be important for the elucidation of the types of damage or responses that might be expected to occur at lower O_3 concentrations, the most relevant data on the genotoxicity of O_3 should be obtained from investigations where the O_3 concentration did not exceed 1960 $\mu g/m^3$ (1 ppm). Research conducted at or below 1 ppm of O_3 will be presented below (See Table 9-24).

Several investigators have examined the in vivo cytogenetic effects of O_3 in rodents and human subjects. Until the reports of Zelac et al. (1971a,b), the toxic effects of O_3 were generally assumed to be confined to the tissues directly in contact with the gas, such as the respiratory epithelium. Due to the highly reactive nature of O_3, little systemic absorption was predicted. Zelac, however, reported a significant increase in chromosome aberrations in peripheral blood lymphocytes from Chinese hamsters exposed to 392 $\mu g/m^3$ (0.2 ppm) for 5 hr. Chromosome breaks, defined as the sum of the number of deletions, rings and dicentrics, were scored in lymphocytes collected immediately after O_3 exposure and at 6 and 15 days postexposure. At all sampling times, there was an increase in the break frequency (breaks/ cell) in the O_3-exposed animals when compared with nonexposed control animals. Zelac et al. (1971b) reported that O_3 was additive with radiation in the production of chromosome breaks. Both O_3 and radiation produced chromosome breaks independently of each other. Simultaneous exposure to 0.2 ppm of O_3 for 5 hr and 230 rad of radiation resulted in the production of 40 percent more breaks than were expected from either agent alone and 70 percent of the total number of breaks expected from the combined effects of the two agents, if it was assumed that the effects were additive.

Chaney (1981) investigated the effects of O_3 exposure on mouse peritoneal exudate cells (peritoneal macrophages) stimulated by an i.p. injection of glycogen. Mice were subsequently exposed by inhalation to 1960 or 9800 $\mu g/m^3$ (1 or 5 ppm) of O_3 for 24 hr. A significant reduction in the average molecular weight of the DNA was observed in the peritoneal exudate cells from mice exposed to 9800 $\mu g/m^3$ (5 ppm) of O_3 but not in animals exposed to 1960 $\mu g/m^3$ (1 ppm) of O_3 for 24 hr. The reduction in the average molecular weight of DNA in O_3-exposed animals indicated the induction of single-strand breaks in the DNA. It should be noted, however, that the alkaline sucrose gradient method of determining the average molecular weight of the DNA does not discriminate between the frank strand-breaks in DNA, produced as a direct effect of the O_3 treatment, and the induction of alkaline-labile lesions in DNA by O_3, which

TABLE 9-24. MUTATIONAL EFFECTS OF OZONE

O_3 concentration		Measurement[a] method	Exposure duration and protocol[b]	Observed effect(s)	Species	Reference
μg/m³	ppm					
196	0.1	MAST	60 min (2.1 ml/min)	Various mutated, growth factor autotrophic states, were recovered; mutant strains differed from parental strains in sensitivity to UV light and excessive production of capsular polysaccharide.	Escherichia coli (MQ 259)	Hamelin and Chung, 1975a
58,800	30	NBKI	3 hr	Induction of a dominant lethal mutation during stages of spermatogenesis; sperm were found to be twice as sensitive as earlier stages.	Drosophila virilis	Erdman and Hernandez, 1982
98,000	50	UV	30 min (2.1 ml/min)	Radiation-sensitive mutant strains defective in repair of single strand (rad 51) and double strand (rad 52) DNA breaks were more sensitive to O_3 cell killing than either the wild-type or the UV light repair deficient strain (rad 3); recombinational repair-deficient strain (rad 6) was moderately sensitive to O_3.	Saccharomyces cerevisae	Dubeau and Chung, 1979
98,000	50	UV	30 min	Induction of forward mutations at 2 loci of adenine biosynthesis (strain C16-11C); induction of reversion mutation at 6 genetic loci (strain XV 185-14C); induction of intragenic and intergenic recombinational mutants (strain D7); O_3 was 20-200x less mutagenic than equitoxic exposures to UV light, x-rays, or MNNG.	Saccharomyces cerevisae	Dubeau and Chung, 1982

[a] Measurement method: MAST = KI-coulometric (Mast meter); NBKI = neutral buffered potassium iodide; UV = UV photometry

[b] O_3 flow rates given in (ml/min), where available.

would be converted to strand breaks under the alkaline condition of the assay. Although these experiments do not prove that O_3 exposure can cause strand breaks in DNA, they do indicate an O_3 effect on DNA at 9800 $\mu g/m^3$ (5 ppm) of O_3 for 24 hr.

Because of the importance of the reports by Zelac et al. (1971a,b) that indicated that significant levels of chromosome aberrations in Chinese hamster peripheral blood lymphocytes collected as late as 15 days after O_3 exposure by inhalation, Tice et al. (1978) tried to repeat the experiments of Zelac as closely as possible. Chinese hamsters were exposed to 843 $\mu g/m^3$ (0.43 ppm) of O_3 by inhalation for 5 hr. The authors investigated chromatid and chromosome aberrations in peripheral blood lymphocytes and bone marrow of control and O_3-exposed animals immediately after exposure and at 7 and 14 days after O_3 exposure. They also investigated the sister chromatid exchange (SCE) frequency in peripheral blood lymphocytes of Chinese hamsters. In separate experiments, SCE frequencies in C57/Bl mice exposed to 2 ppm of O_3 for 6 hr were examined in peripheral blood cells collected from the animals immediately after O_3 exposure and at 7 and 14 days after O_3 exposure.

The authors reported no significant increase in the SCE frequency of the O_3-exposed hamsters or mice at any sampling time, nor did they observe a significant increase in the number of chromosome aberrations of phytohemagglutinin (PHA)-stimulated peripheral blood or bone marrow cells. The only reported statistically significant differences were observed in peripheral blood lymphocytes, in which there was an increase in the number of chromatid deletions and achromatic lesions in the 7- and 14-day samples, respectively. Both types of chromatid aberration were observed at consistently higher frequencies in the blood samples of the O_3-exposed animals, frequently in the range of 50 to 100 percent increases over the control values. Statistically significant differences were assigned at the 1 percent level of significance. It is not clear how a slightly more rigorous evaluation of significance (e.g., $p < 0.05$) would have influenced the interpretation of the data.

Although both Zelac et al. (1971a,b) and Tice et al. (1978) reported significant increases in chromosome aberrations in peripheral blood lymphocytes following 5-hr exposures to 392 to 843 $\mu g/m^3$ (0.2 to 0.4 ppm) of O_3, the types of lesions observed in the two studies were clearly different. Tice et al. observed chromatid-type lesions and no increase in the chromosome aberrations, whereas Zelac et al. reported a significant increase in the number of chromosome aberrations. There were a number of differences in the experimental protocols that may have produced the seemingly different results:

1. The animals were exposed to different concentrations of O_3. Zelac et al. (1971a) administered 470 to 590 $\mu g/m^3$ (0.24 to 0.3 ppm) of O_3 to Chinese hamsters for 5 hr, whereas Tice et al. (1978) exposed animals to an atmosphere of 840 $\mu g/m^3$ (0.43 ppm) of O_3 for 5 hr.
2. Zelac stimulated peripheral blood lymphocytes into DNA synthesis with pokeweed mitogen, which is mainly a B-lymphocyte mitogen. In the experiments of Tice et al. (1978), lymphocytes were stimulated with PHA, which is a T-lymphocyte mitogen (Ling and Kay, 1975).
3. Zelac cultured lymphocytes with the mitogen *in vitro* for 3 days (72 hr), whereas Tice et al. cultured lymphocytes with mitogen for 52 hours.

Because of the longer incubation time with the mitogen in the experiments of Zelac et al. (1971a), lymphocytes may have converted chromatid type aberrations, like those reported by Tice et al. (1978), into chromosome aberrations with another round of DNA synthesis. Because the experiments of Zelac et al. and Tice et al. were conducted with peripheral blood lymphocytes stimulated by two different mitogens (pokeweed vs. PHA), the cytogenetic consequences of O_3 exposure were examined in different populations of lymphocytes. If one of the populations of lymphocytes was more sensitive to O_3 than the other, different cytogenetic responses could be expected when PHA was used as a mitogen, compared with the results with the use of polkweed mitogen.

There is evidence that the B-lymphocyte may be more sensitive to O_3 than the T-lymphocyte. Savino et al. (1978) measured the effects of O_3 on human cellular and humoral immunity by measuring rosette formation with human lymphocytes (See Chapter 10). Rosette formation measures the reaction of antigenic red cells with surface membrane sites on lymphocytes. Different antigenic RBCs are used to distinguish T-lymphocytes from B-lymphocytes. Rosette formation with B-lymphocytes was significantly depressed in eight human subjects exposed to 784 $\mu g/m^3$ (0.4 ppm) of O_3 by inhalation for 4 hr. A similar inhibition of rosette formation was not observed with T-lymphocytes from the same subjects. The depressed B-cell responses persisted for 2 weeks after O_3 exposure, although partial recovery to the pre-exposure level was evident.

It cannot be stated with any certainty how the differences in the O_3 exposure, the choice of mitogen, and the length of the mitogen exposure may have contributed to the differences in the results reported by Zelac et al. (1971a,b) and by Tice et al. (1978). There are sufficient differences in the experimental protocols of the two reports so that the results need not be considered directly contradictory.

An assumption that is made in all of the reports in which lymphocytes are stimulated with mitogens is that the lymphocytes from the O_3-exposed animals and the control animals are equally sensitive to the mitogenic stimulus. This assumption is probably not correct, because in investigations by Peterson et al. (1978a,b) the proliferation of human lymphocytes exposed to PHA was significantly suppressed in blood samples taken immediately after the subjects were exposed to 784 $\mu g/m^3$ (0.4 ppm) of O_3 for 4 hr (See 10.7). Other reports have suggested that O_3 might inhibit or inactivate the PHA receptor on lymphocytes (see Gooch et al., 1976). Because the ability to measure chromosome aberrations in mitotically arrested cells is absolutely dependent on the induction of G_0 or G_1 cells into the cycling state, cells exposed to sufficient concentrations of O_3 would not be stimulated to divide, and hence no O_3-induced cytogenic effects would be observed in activated cells. In their report, Tice et al. (1978) stated that the lymphocytes of the O_3-exposed animals in their experiments "did tend to be worse than those from controls." Only a small difference in the number of responding lymphocytes could make large differences in the results of the experiments if O_3-damaged lymphocytes were selected against in the cytogenetic investigations.

In other investigations with rodents, Gooch et al. (1976) analyzed bone marrow samples from Chinese hamsters exposed to 451 $\mu g/m^3$ (0.23 ppm) of O_3 for 5 hr. Marrow samples were taken at 2, 6, and 12 hr following O_3 exposures. In separate experiments, male C_3H mice were exposed to 294 or 412 $\mu g/m^3$ (0.15 or 0.21 ppm) of O_3 for 5 hr, or to 1940 $\mu g/m^3$ (0.99 ppm) of O_3 for 2 hr. Blood samples were drawn from these animals at various times for up to 2 weeks following O_3 exposure. The mice were killed 8 weeks following O_3 exposure, and spermatocyte preparations were made and analyzed for reciprocal translocations. Data from the Chinese hamster bone marrow samples and the mouse leukocytes indicated that there was no effect induced by O_3 treatment on the frequency of chromatid or chromosome aberrations, nor were there any recognizable reciprocal translocations in the primary spermatocytes.

Several investigators have examined the effects of O_3 on human cells in vitro. Fetner (1962) observed the induction of chromatid deletions in human KB cells exposed to 15,680 $\mu g/m^3$ (8 ppm) of O_3 for 5 to 25 min. Sweet et al. (1980) reported that the growth of human cells from breast, lung, and uterine tumors was inhibited by exposure to 588 to 1568 $\mu g/m^3$ (0.3 to 0.8 ppm) of O_3 for 8 days in culture.

Guerrero et al. (1979) performed SCE analysis on diploid human fetal lung cells (WI-38) exposed to 0, 490, 980, 1470, or 1960 $\mu g/m^3$ (0, 0.25, 0.5, 0.75, or 1.0 ppm) O_3 for 1 hour in vitro. A dose-related increase in the SCE frequency was observed in the WI-38 human fibroblasts exposed to O_3. In the same report, the authors stated that no significant increase in the SCE frequency over control values was observed in peripheral blood lymphocytes from subjects exposed to O_3 by inhalation (Chapter 10). Unless the lymphocyte is intrinsically less sensitive to the induction of SCE by O_3 than the WI-38 human fetal lung fibroblast, the results indicate that exposure of human subjects to 980 $\mu g/m^3$ (0.5 ppm) of O_3 for 2 hr did not result in a sufficiently high concentration of O_3 or O_3 reaction products in the circulation to induce an increase in the SCE frequency in the lymphocytes. From the authors' data on the induction of SCE in WI-38 cells, the concentration of O_3 required to induce SCE in human cells is approximately 490 $\mu g/m^3$ (0.25 ppm) for 1 hour.

Gooch et al. (1976) also investigated the effects of O_3 exposure on human cells in vitro. In these experiments, lymphocytes were stimulated with PHA for 12 or 36 hr before the O_3 exposure to obviate the potential problems of O_3 inactivation of the PHA receptor. Human leukocyte cultures were exposed to 3920 $\mu g/m^3$ (2 ppm) of O_3 for various lengths of time to accumulate total O_3 exposure doses of 3234 to 27,832 $\mu g/m^3$ per hour (1.65 to 14.2 ppm/hr). The results showed no increase in the chromatid and chromosome aberrations at any total dose, with the possible exception of an apparent spike in chromatid aberrations at a total exposure of 14,170 $\mu g/m^3$ (7.23 ppm/hr). The significance of this observation is unclear because the data showed no dose-response increase in the number of chromatid aberrations at concentrations near 7.25 ppm/hr, and the authors did not report how, or indeed if, the data were statistically evaluated for differences in chromatid aberrations.

In summary, in vitro O_3 exposure has been shown to produce toxic effects on cells and cellular components including the genetic material. Cytogenetic toxicity has been reported in cells in culture and in cells isolated from animals if O_3 exposure has occurred at sufficiently high levels and for sufficiently long periods.

9.4.5.2 Mutational Effects of Ozone. The mutagenic effects of O_3 have been investigated in surprisingly few instances (Table 9-24). No publication to date has investigated the mutagenic effects of O_3 in mammalian cells.

Sparrow and Schairer (1974) measured an increase in the frequency, over the background level, in the induction of pink or colorless mutant cells or groups of cells in petal and/or stamen hairs of mature flowers of various blue-flowered *Tradescantia*. No O_3 concentration was reported in this publication.

E. coli, strain MQ 259, were mutated to various growth factor auxotrophic states, including requirements for most common amino acids, vitamins, and purines and pyrimidines (Hamelin and Chung, 1975a). Ozonated air (196 $\mu g/m^3$, 0.1 ppm) was passed through the bacterial suspensions at a rate of 2.1 L/min for 30 min. Many of the O_3-induced mutant strains were either more or less sensitive to UV light than the parental strain. Other mutant strains, called mucoid mutants, had apparent defects in DNA repair pathways and were characterized (Hamelin and Chung, 1975b) as producing excessive amounts of capsular polysaccharide.

Erdman and Hernandez (1982) investigated the induction of dominant lethal mutations in *Drosophila virilis* exposed to 58,800 $\mu g/m^3$ (30 ppm) of O_3 for 3 hr. O_3 induced dominant lethal mutations at various stages of spermatogenesis. The sperm-sperm bundle stage was the most sensitive to O_3, and the meiotic cells were the least sensitive.

Dubeau and Chung (1979, 1982) have investigated the mutagenic and cytotoxic effects of O_3 on *Saccharomyces cerevisae*. Several different strains were utilized to investigate forward, reverse, and recombinational mutations. ozone (98,000 $\mu g/m^3$, 50 ppm; 30 to 90 min) induced a variety of forward and reverse mutations as well as gene conversion and mitotic crossing-over. Both base-substitution and frame-shift mutations were induced by O_3. Ozone was shown to be more recombinogenic than mutagenic in yeast, probably as a result of the induction of strand breaks in DNA, either directly or indirectly.

In the investigation of Dubeau and Chung (1982), the mutagenic potency of O_3 (98,000 $\mu g/m^3$, 50 ppm; 30 to 90 min) was compared with other known mutagens. The positive controls were UV light (1.54 J/m^2 per second, 1-min exposure), N-methyl-N'-nitrosoguanidine (MNNG) (50 $\mu g/mL$, 15 min), or x-rays (2 kR/min, 40 min). By comparing the induced mutation frequency at similar cellular survival levels for O_3, MNNG, UV light, and x-rays, it was shown that O_3 was a very weak mutagen. Induced mutation frequencies were generally 20 to 200 times lower for O_3 than for the other three mutagens.

In summary, the mutagenic properties of O_3 have been demonstrated in procaryotic and eucaryotic cells. Only one study, however, (Hamelin and Chung, 1975a, with E. coli) investigated the mutagenic effect of O_3 at concentrations of less than 1 ppm. The results clearly indicate that if cells in culture are exposed to sufficiently high concentrations of O_3 for sufficiently long periods, mutations will result. The relevance of the presently described investigations to human or even other mammalian mutagenicity is not apparent. Additional studies with human and other mammalian cells will be required before the mutagenic potency of O_3 toward these species can be determined.

9.4.6 Other Extrapulmonary Effects

9.4.6.1 Liver. A series of studies reviewed by Graham et al. (1983a) have shown that O_3 increases drug-induced sleeping time in animals (Table 9-25). The animal was injected with the drug (typically pentobarbital), and the time to the loss of the righting reflex and the sleeping time (time between loss and regaining of the righting reflex) were measured. Because the time to the loss of the righting reflex was very rarely altered in the experiments described below, it will not be discussed further. Animals awake from pentobarbital-induced sleep, because liver xenobiotic metabolism transforms the drug into an inactive form. Therefore, this response is interpreted as an extrapulmonary effect.

Gardner et al. (1974) were the first to observe that O_3 increases pentobarbital-induced sleeping time. Female CD-1 mice were exposed for 3 hr/day for up to 7 days to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 and the increase was found on days 2 and 3 of exposure, with the greatest response occurring on day 2. Complete tolerance did not occur; when mice were pre-exposed (1960 $\mu g/m^3$, 1.0 ppm; 3 hr/day for 7 days) and then challenged with a 3-hr exposure to 9800 $\mu g/m^3$ (5.0 ppm) on the eighth day, pentobarbital-induced sleeping time increased greatly.

A series of follow-up studies was conducted to characterize the effect further. To evaluate female mouse strain sensitivity, one outbred (CD-1) and two inbred (C57BL/6N and DBA/2N) strains were compared (Graham et al., 1981).

TABLE 9-25. EFFECTS OF OZONE ON THE LIVER

Ozone concentration µg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effects(s)	Species	Reference
196- 9800	0.1- 5	CHEM GPT	3 hr/day, 1-17/days	Increase in pentobarbital-induced sleeping time with following exposure regimens; 0.1 ppm, 15 or 16 days; 0.25 ppm, 6 or 7 days; 0.5 ppm, 2 or 3 days; 1 ppm, 1, 2, or 3 days; 5 ppm, 1 day. No effects at days before or after days given above.	Mouse (female)	Graham et al., 1981
588	0.3	UV	3 hr	No effect on liver reduced ascorbic acid levels.	Rat	Calabrese et al., 1983c
1470 5880 19,600	0.75 3 10	NBKI	3 hr	No effect on hepatic benzo(a)pyrene hydroxylase activity.	Hamster	Palmer et al., 1971
1600 (max)	0.82 (max)	UV	4 hr (max)	Decrease in hepatic reduced ascorbic acid content. Actual exposure regimens not reported, only maximal levels given.	Mouse	Veninga et al., 1981
1960	1	ND	3 hr/day	Increase in pentobarbital-induced sleeping time after 2 or 3 days, but not other days (up to 7 days). No tolerance to a challenge of O_3 (9800 µg/m³, 5 ppm x 3 hr) to mice pre-exposed to 1 ppm for 7 days.	Mouse (female)	Gardner et al., 1974
1960	1	CHEM GPT	5 hr	Increase in pentobarbital-induced sleeping time in 3 strains of female mice (CD-1, C57BL, and DBA), female rats, and male and female hamsters. Male mice and rats not affected, even when male mice received 3 days (5 hr/day) of exposure. The increase in male hamsters was less than the increase in female hamsters.	Mouse, rat, hamster (male and female)	Graham et al., 1981

TABLE 9-25. EFFECTS OF OZONE ON THE LIVER (continued)

Ozone concentration μg/m^3	ppm	Measurement^{a,b} method	Exposure duration and protocol	Observed effects(s)	Species	Reference
1960	1	CHEM GPT	5 hr/day, 1,2,3 or 4 days	Increase in pentobarbital-induced sleeping time at 1,2, and 3 days, decreased with increasing days of exposure. 24-hr postexposure for each group, no effects occurred.	Mouse (female)	Graham et al., 1981
1960	1	CHEM GPT	5 hr	Increase in hexobarbital- and thiopental-induced sleeping time and zoxazolamine-induced paralysis time. Pretreatment with mixed function oxidase inducers (phenobarbital, pregneolone-16a-carbonitrile, and β-naphthoflavone, but not pentobarbital) decreased phenobarbital-induced sleeping time in CD-1 mice, and O_3 increased the sleeping time in all groups. Pretreatment with inhibitors (SF525A, piperonyl butoxide) reduced the sleeping time, but O_3 increased the sleeping time, with the magnitude of the increase becoming larger as the dose of inhibitor was increased.	Mouse (female)	Graham et al., 1982a
1960 9800	1 5	CHEM GPT	5 hr 3 hr	No effect on hepatic cytochrome P-450 concentration, aminopyrine N-demethylase, or p-nitroanisole O-demethylase activities. Aniline hydroxylase activity increased at 5 ppm (3 hr) and at 1 ppm (5 hr/day x 2 days). No change in liver to body weight ratios.	Mouse (female)	Graham et al., 1982b

TABLE 9-25. EFFECTS OF OZONE ON THE LIVER (continued)

Ozone concentration μg/m³	Ozone concentration ppm	Measurement[a,b] method	Exposure duration and protocol	Observed effects(s)	Species	Reference
1960 9800	1 5	CHEM GPT	5 hr	At 1 ppm: 71% increase in plasma half-life of pentobarbital, decrease ($p = 0.06$) in slope of clearance curve. At 5 ppm: 106% increase in plasma half-life of pentobarbital; decrease in slope of clearance curve; no effect on concentrations of pentobarbital in brain at time of awakening; no change in type of pentobarbital metabolites in serum/or brain.	Mouse (female)	Graham 1979; Graham et al., 1983, 1985
1960	1	ND	90 min	No effect on hepatic cytochrome P-450 concentration.	Rabbit	Goldstein et al., 1975
3920	2	UV	8 hr/day	Supplementing or depriving rats of vitamin E or selenium altered the O_3 effect. O_3 caused changes in several *in vitro* enzyme activities in the liver and kidney (see text).	Rat	Reddy et al., 1983

[a] Measurement method: CHEM = gas-phase chemiluminescence; NBKI = neutral buffered potassium iodide; UV = UV photometry; ND = not described

[b] Calibration method: GPT = gas phase titration

Ozone (1960 μg/m^3, 1.0 ppm for 5 hr) increased pentobarbital-induced sleeping time in all strains. To determine whether this effect was sex- or species-specific, male and female CD-1 mice, rats, and hamsters were exposed for 5 hr to 1960 μg/m^3 (1.0 ppm) of O_3 (Graham et al., 1981). The females of all species exhibited an increased pentobarbital-induced sleeping time. Male mice and rats were not affected. Male hamsters had an increase in sleeping time, but this increase was less ($p = 0.075$) than the increase observed in the females. Thus, the effect is not specific to strain of mouse or to three species of animals, but it is sex-specific, with females being more susceptible. Female CD-1 mice were exposed to 196 to 9800 μg/m^3 (0.1 to 5.0 ppm) of O_3 for 3 hr/day for a varying number of days (Graham et al., 1981). At 1960 μg/m^3 (1.0 ppm), effects were observed after 1, 2, or 3 days of exposure, with the largest change occurring on day 2. At 980 μg/m^3 (0.5 ppm), the greatest increase in pentobarbital-induced sleeping time was observed on day 3, but at 490 μg/m^3 (0.25 ppm), 6 days of exposure were required to cause an increase. At the lowest concentration evaluated (196 μg/m^3, 0.1 ppm), the increase was only observed at days 15 and 16 of exposure. Thus, as the concentration of O_3 was decreased, increasing numbers of daily 3-hr exposures were required to significantly increase pentobarbital-induced sleeping time. Once the maximal effect occurred, increasing the number of exposures resulted in a diminution of the effect. Generally, effects were observed around an approximate C X T (concentration, ppm x time, hr) value of 5. Also, CD-1 female mice were exposed to four different concentrations of O_3 (1960 to 196 μg/m^3, 1.0 to 0.1 ppm) for 5 to 20 hr continuously in a fashion yielding a C x T value of 5 (Graham et al., 1981). All regimens increased pentobarbital-induced sleeping time. The time to recovery was examined in mice exposed to 1960 μg/m^3 (1.0 ppm) of O_3 for 5 hr/day. Recovery was complete within 24 hr after exposure, whether exposure was for 1, 2, or 3 days.

To determine whether the previous responses were specific to pentobarbital, other drugs with known and different mechanisms for termination of action were used in CD-1 female mice exposed to 1960 μg/m^3 (1.0 ppm) of O_3 for 5 hr (Graham et al., 1982a). Ozone increased sleeping time induced by hexobarbital and thiopental and paralysis time induced by zoxazolamine. Within the liver, pentobarbital and hexobarbital metabolism is more related to cytochrome P-450 than to cytochrome P-448-dependent activities. Zoxazolamine metabolism is more related to cytochrome P-448 than to cytochrome P-450.

Other major differences in the nature of the metabolism (i.e., aliphatic vs. aromatic) also exist between these three drugs. In contrast to the above, sedation from thiopental is terminated because of drug redistribution. Thus, it would appear that O_3 might affect some aspects of both drug redistribution and metabolism. Although there are different mechanisms involved in hexobarbital, zoxazolamine, and pentobarbital metabolism, it is possible that some common component(s) of metabolism may have been altered.

CD-1 female mice were pretreated with mixed-function oxidase inducers and inhibitors with partially characterized mechanisms of action to relate any potential differences in the effect of O_3 to differences in the actions of the agents. Mice were exposed to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 or air for 5 hr before measurement of pentobarbital-induced sleeping time (Graham et al., 1982a). Again, the effect of O_3 was observed, but mechanisms were not elucidated.

The effect of O_3 on hepatic mixed-function oxidases in CD-1 female mice was evaluated in an attempt to relate to the sleeping time studies (Graham et al., 1982b). A 3-hr exposure to concentrations of O_3 as high as 9800 $\mu g/m^3$ (5.0 ppm) did not change the concentration of cytochrome P-450 or the activities of related enzymes (aminopyrine N-demethylase or p-nitroanisole O-demethylase). However, this exposure regimen and another (1960 $\mu g/m^3$, 1.0 ppm, 5 hr/day for 2 days) increased slightly the activity of another mixed-function oxidase, aniline hydroxylase. Goldstein et al. (1975) also found no effect of a 90-min exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 on liver cytochrome P-450 levels in rabbits. Hepatic benzo(a)pyrene hydroxylase (another mixed-function oxidase) activity of hamsters was unchanged by a 3-hr exposure to up to 19,600 $\mu g/m^3$ (10 ppm) of O_3 (Palmer et al., 1971).

Pentobarbital pharmacokinetics in female CD-1 mice were also examined. A 3-hr exposure to 9800 $\mu g/m^3$ (5.0 ppm) of O_3 did not affect brain concentrations of pentobarbital at time of awakening, even though sleeping time was increased (Graham et al., 1985). Therefore, it appears that O_3 did not alter the sensitivity of brain receptors to pentobarbital. A similar exposure regimen also did not alter the pattern of brain or plasma metabolites of pentobarbital at various times up to 90 min postexposure (Graham et al., 1985). Following this exposure, first-order clearance kinetics of pentobarbital were observed in both the air and O_3 groups, and O_3 increased the plasma half-life by 106 percent (Graham et al., 1985). Mice exposed to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 for 5 hr had a 71 percent increase in the plasma half-life of pentobarbital. This ozone exposure resulted in a decrease ($p = 0.06$) in the slope of the clearance curve. Clearance followed first-order kinetics with a one-compartment model in this experiment also.

In summary, the mechanism(s) for the effect of O_3 on pentobarbital-induced sleeping time are not known definitively. However, it is hypothesized (Graham et al., 1983) that some common aspect(s) of drug metabolism is quantitatively reduced, whether it is direct (e.g., enzymatic) or indirect (e.g., liver blood flow) or a combination of both. In addition, drug redistribution is apparently slowed. It is unlikely that ozone itself caused these effects at target sites distant from the lung (see Section 9.2). Because of the free-radical nature of oxidation initiated by O_3, a myriad of oxygenated products, several of which have toxic potential, may be formed in the lung (Section 9.3.3). However, the stability of such products in the blood and their reaction with organs such as the liver are speculative at present.

Reddy et al. (1983) studied the effects of a 7-day (8 hr/day) exposure of rats to 3920 $\mu g/m^3$ (2.0 ppm) of O_3 on liver xenobiotic metabolism by performing in vitro enzyme assays. Although lower O_3 levels were not tested, this study is presented because it indicates the potential of O_3 to cause hepatic and kidney effects. The rats used were either supplemented or deficient in both vitamin E and selenium. Ozone exposure caused a decrease in microsomal cytochrome P-450 hydroperoxidase activity in livers of rats deficient in both substances, whereas an increase resulted in the supplemented animals. Rats deficient in vitamin E and selenium experienced a decrease in liver microsomal epoxide hydrolase activity after O_3 exposure; no effect was observed in supplemented rats. Glutathione S-transferase activity was increased in the liver and kidney in both the supplemented and deficient groups. Selenium-independent glutathione peroxidase activity was not significantly affected in the livers of the supplemented or deficient rats. However, O_3 decreased selenium-dependent glutathione peroxidase activity in the livers of supplemented rats and caused an increase in deficient rats. In the kidney, both these groups of animals had an increase in this enzyme activity. Other groups of rats (deficient in vitamin E, supplemented with selenium; supplemented with vitamin E, deficient in selenium) were examined also and in some cases, different results were observed. The authors interpreted these results (along with pulmonary effects) as a compensatory mechanism to protect cells from oxidants. A more extensive interpretation of the effects depends on the nutritional status and the presence of other compounds metabolized by the affected enzymes. For example, epoxide hydrolase, which was decreased in the vitamin E- and selenium-deficient rats, metabolizes reactive epoxides to dihydrodiols. The metabolism of a substance such as benzo(a)pyrene would be expected to be affected by such a change. However, because of the complexity of the metabolism of a given chemical, such as benzo(a)pyrene, a precise interpretation is not possible at this time.

Veninga et al. (1981) exposed mice to O_3 and evaluated hepatic reduced ascorbic acid content. The authors expressed the exposure regimen in the form of a C x T value from about 0.2 to 3.2. Actual exposure regimes cannot be determined. They stated that the maximal O_3 level was 1600 $\mu g/m^3$ (0.82 ppm) and the maximal exposure time was 4 hr, which would have resulted in a C x T of about 3.2. Animals were studied at 0, 30, and 120 min postexposure. It appeared that immediately after exposure, a C x T value $<$ 0.4 caused a decrease in the reduced ascorbic acid content of the liver. At a C x T value of 0.4 and 0.8, there appeared to be an increase that was not observed at higher values. For the 30-min postexposure groups, the increase in reduced ascorbic acid shifted, with the greatest increase being at about a C x T value of 1.2 and no change occurring at a C x T value of 2.0. The 120-min postexposure group was roughly similar to the immediate post-exposure group. No effects occurred 24 hr postexposure.

Hepatic reduced ascorbic acid levels were also studied by Calabrese et al. (1983c) in rats exposed for 3 hr to 588 $\mu g/m^3$ (0.3 ppm) of O_3 and examined at 5 postexposure periods up to 24 hr. Rats had significantly increased ascorbic acid levels in both the O_3 and air groups, with the greater change taking place in the air group. Thus, there were no changes due to O_3. Likewise, there was no O_3 effect on reduced ascorbic acid content in the serum.

9.4.6.2 The Endocrine System. A summary of the effects of O_3 on the endocrine system, gastrointestinal tract, and urine is given in Table 9-26. Fairchild and co-workers were the first to observe the involvement of the endocrine system in O_3 toxicology. Mice exposed to 11,368 $\mu g/m^3$ (5.8 ppm) for 4 hr were protected against mortality by α-naphthylthiourea (ANTU) (Fairchild et al., 1959). Because ANTU has antithyroidal activity and can alter adrenal cortical function, Fairchild and Graham (1963) hypothesized a possible interaction of O_3 with the pituitary-thyroid-adrenal axis. In exploring the hypothesis, they exposed mice and rats for 3 to 4 hr to unspecified lethal concentrations of O_3. Thyroid-blocking agents and thyroidectomy increased the

TABLE 9-26. EFFECTS OF OZONE ON THE ENDOCRINE SYSTEM, GASTROINTESTINAL TRACT, AND URINE

Ozone concentration		Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
μg/m³	ppm					
5.4 21 110	0.003 0.01 0.056	c	93 days, continuous	From 6th wk to end of exposure, 0.056 ppm increased the urine concentration of 17-ketosteroids. After 93 days of exposure to 0.056 ppm, the ascorbic and level of the adrenal glands was decreased. No data were presented for these effects.	Rat	Eglite, 1968
490 2940	0.25 1.5	d	2 hr 30 min	1.5 ppm of O_3 (30 min) inhibited gastric mortality; recovery was rapid. The lower level caused no effects.	Rat	Roth and Tansy, 1972
980 1960	0.5 1	I	5 hr/day, 4 days	No effects on thyroid release of ^{131}I, 96-384 hr post ^{131}I injection.	Rat	Fairchild et al., 1964
1470	0.75	ND	4-8 hr	LM and TEM changes in parathyroid glands. Loss of "clusterlike" arrangement of parenchyma. Dilated capillaries. Vacuolated chief cells. Increased RER, prominent Golgi, abundant secretory granules.	Rabbit	Atwal and Wilson, 1974
1470	0.75	ND	48 hr postexposure 1-20 days	Early postexposure parathyroid glands enlarged and congested with focal vasculitis. After 7 days postexposure, parenchymal atrophy, leukocyte infiltration and capillary proliferation. Authors suggest lesions may be due to autoimmune reactions.	Rabbit	Atwal et al., 1975
1470	0.75	ND	48 hr postexposure 12-18 days	Microvascular changes in the parathyroid glands, including hemorrhage, endothelial proliferation, platelet aggregation, and lymphocyte infiltration.	Dog	Atwal and Pemsingh, 1981
1470	0.75	ND	48 hr, 3, 10-13, 18 days post-exposure	Ciliated cysts found in parathyroid gland. Mallory body-like inclusions found in chief cells of parathyroid with highest incidence being 10-13 days postexposure.	Dog	Pemsingh and Atwal, 1983 Atwal and Pemsingh, 1984
1568-2940 (range)	0.8-1.5 (range)	NBKI	6 hr/day, 4 days/wk about 19 wk	Lower titratable acidity of urine, with no changes in levels of creatine, uric acid/creatinine, amino acid nitrogen/creatinine, or excretion of 12 amino acids.	Rat	Hathaway and Terrill, 1962

TABLE 9-26. EFFECTS OF OZONE ON THE ENDOCRINE SYSTEM, GASTROINTESTINAL TRACT, AND URINE (continued)

Ozone concentration μg/m³	Ozone concentration ppm	Measurement[a] method	Exposure duration and protocol	Observed effect(s)[b]	Species	Reference
1960 3920 7840	1 2 4	I	5 hr	Decreased release of ^{131}I from thyroid, 48-384 hr post ^{131}Injection to all O_3 levels above 1 ppm.	Rat	Fairchild et al., 1964
1960	1	ND	24 hr	Decreased serum level of thyroid-stimulating hormone from anterior pituitary, thyroid hormones (T_3, T_4, and free T_4), and protein-bound iodine; no change in unsaturated binding capacity of thyroid-binding globulin in serum; increase in prolactin levels; no change in levels of corticotropin, growth hormone, luteinizing hormone, follicle stimulating hormone from pituitary or insulin. Thyroidectomy prevented the effect on TSH levels. There was no effect on the circulating half-life of ^{131}I-TSH. The anterior pituitaries had fewer cells, but more TSH/cell. The thyroid gland was also altered. Exposures to between 0.2 to 2 ppm for unspecified lengths of time up to a potential maximum of 500 hr also caused a decrease in TSH levels.	Rat	Clemons and Garcia, 1980a,b.
1960	1	ND	24 hr	Decreased serum levels of T_3, T_4, and TSH. In thyroidectomized and hypophysectomized rats, the decrease in T_4 was greater when rats were supplemented with T_4 in the drinking water.	Rat	Clemons and Wei, 1984
79,800	> 5.0	I	> 3 hr < 8 hr	Anti-thyroid agents, thyroidectomy, hypophysectomy, and adrenalectomy protected against O_3-induced mortality. Injection of thyroid hormones decreased survival after O_3 exposure.	Mouse, rat	Fairchild et al., 1959; Fairchild and Graham, 1963; Fairchild, 1963.
9800	5	CHEM	3 hr	Increased levels of 5-hydroxytryptamine in lung; decreases in brain; no change in kidney.	Rat	Suzuki, 1976.

[a] Measurement method: CHEM = gas phase chemiluminescence; NBKI = neutral buffered potassium iodide; I = iodometric (Byers and Saltzman, 1959); ND = not described.

[b] Abbreviations used: LM = light microscopy; TEM = transmission electron microscopy; RER = rough endoplasmic reticulum; T_3 = triiodothyronine; T_4 = thyroxine.

[c] Spectrophotometric technique (dihydroacridine).

[d] Flow rates from ozonator.

survival of mice and rats acutely exposed to O_3, and injections of the thyroid hormones, thyroxine (T_4), or triiodothyronine (T_3) decreased their survival. This response in animals with altered thyroid function was not specific to a concomitant altered metabolic rate, because another drug (dinitrophenol), which increases metabolism, had no effect on the O_3 response.

Hypophysectomy and adrenalectomy also protected against O_3-induced mortality, presumably in rodents (Fairchild, 1963). Hypophysectomy would prevent the release of thyroid-stimulating hormone, thereby causing a hypothyroid condition as well as preventing the release of adrenocorticotropic hormone (ACTH), which would cause a decreased stimulation of the adrenal cortex to release hormones. This confirms the above-mentioned finding of thyroid involvement in O_3 toxicity and suggests that a decrease in adrenocorticosteroids reduces O_3 toxicity. Rats that have been adrenalectomized and treated with adrenergic blocking agents are more resistant to O_3 than rats that have only been adrenalectomized, indicating that decreases in catecholamines reduce O_3 toxicity.

Potential tolerance to the effect of O_3 on thyroid activity was also investigated by Fairchild et al. (1964). A variety of exposure regimens were used for the rats, and the release of ^{131}I was used as an index of thyroid function. A 5-hr exposure to 1960, 3920, or 7840 $\mu g/m^3$ (1, 2, or 4 ppm) of O_3 inhibited the release of ^{131}I at several time periods post injection of ^{131}I (48, 96, 192, and 384 hr). The rats were injected before the 5-hr exposure, presumably shortly before exposure. Twenty-four hr post-injection, only the highest O_3 concentration showed an effect. Rats were also exposed for 5 hr/day for 4 days to either 980 or 1960 $\mu g/m^3$ (0.5 or 1.0 ppm) of O_3. At 96, 192, and 384 hr post-injection of ^{131}I, no effects were observed. Thus, tolerance appeared to have occurred, a finding consistent with lethality studies with O_3 (Matzen, 1957a). In another study, rats were exposed to 3920 $\mu g/m^3$ (2.0 ppm) of O_3 5 hr/day for 2 days and challenged with a 5-hr exposure to 7840 $\mu g/m^3$ (4.0 ppm) on the third day. These animals exhibited a greater effect than rats that received only the 7840-$\mu g/m^3$ (4.0 ppm) challenge. This difference persisted for 48, 96, and 192 hr post-injection. Thus, although it appears that tolerance occurs, it results in a condition that leads to stimulation of thyroid activity after a subsequent exposure to an O_3 challenge.

Clemons and Garcia (1980a,b) extended this area of research by investigating the effects of O_3 on the hypothalamo-pituitary-thyroid axis of rats. Generally, these three endocrine organs regulate the function of each other through complex feedback mechanisms. Either stimulation or inhibition of the hypothalamus regulates the release of thyrotropin-releasing hormone (TRH). The thyroid hormones (T_3 and T_4) can stimulate TRH. Thyrotropin-releasing hormone and circulating thyroid hormones (T_3 and T_4) regulate secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary. Stimulation of the thyroid by TSH releases T_3 and T_4. A 24-hr exposure to 1960 $\mu g/m^3$ (1.0 ppm) of O_3 caused decreases in the serum concentrations of TSH, T_3, T_4, free T_4, and protein-bound iodine. There was no change in the uptake of T_3, and thus no change in the unsaturated binding capacity of thyroid-binding globulin in the serum. Prolactin levels were increased also, but no alterations were observed in the concentrations of other hormones (corticotropin, growth hormone, luteinizing hormone, follicle-stimulating hormone, and insulin). Plasma TSH was also evaluated after continuous exposures to between 392 and 3920 $\mu g/m^3$ (0.2 and 2.0 ppm) for unspecified lengths of time in a fashion to result in a concentration x time relationship between about 2 and 100. Plasma TSH was decreased after a C x T exposure of about 6. These data cannot be independently interpreted, because the specific exposure regimens were not given. The authors state, without any supporting data, that the decrease in TSH levels persisted "beyond two weeks" when exposure was continued. Therefore, it appears that tolerance may not have occurred. Thyroidectomized rats exposed to 1960, 3920, 5880 or 7840 $\mu g/m^3$ (1.0, 2.0, 3.0, or 4.0 ppm) of O_3 for 24 hr did not exhibit a decrease in the levels of TSH. Exposure to (presumably) 1960 $\mu g/m^3$ (1.0 ppm) for 24 hr did not alter the circulating half-life of ^{125}I-labeled TSH injected into the rats, and therefore, there apparently is no effect on TSH once it is released from the pituitary.

To evaluate pituitary function further, rats exposed 24 hr to 1960 $\mu g/m^3$ (1.0 ppm) of ozone were immediately subjected to a 45-min exposure to the cold (5°C) (Clemons and Garcia, 1980a,b). The anterior pituitary released an increased level of TSH, indicating that the hypothalamus was still able to respond (via increased TRH) after O_3 exposure. The increase was greater in the O_3 group, which might indicate increased production of TRH or increased sensitivity to TRH. In addition, the anterior pituitaries of the O_3 group had fewer cells than the air group. The cells from the O_3-exposed rats had more TSH and prolactin per 1000 cells, irrespective of whether the cells had received a TRH treatment. The cells from the O_3 group also released a greater amount of TSH, but not prolactin, into the tissue culture medium.

The thyroid gland itself was altered by the O_3 exposure (apparently 1960 $\mu g/m^3$, 1.0 ppm, for 24 hr). Ozone increased thyroid weight without changing protein content (e.g., edema) and decreased the release of T_4 per milligram of tissue. There was no change in T_4 release per gland. Clemons and Garcia (1980a,b) interpreted these findings as an O_3-induced lowering of the hypothalamic set point for the pituitary-thyroid axis and a simultaneous reduction of prolactin-inhibiting-factor activity in the hypothalamus.

The effect of a 24-hr exposure to 1960 $\mu g/m^3$ (1 ppm) O_3 on exogenous T_4 levels was examined in rats by Clemons and Wei (1984) to increase understanding of their previous studies. Normal, thyroidectomized, and hypophysectomized rats were exposed to O_3 and serum T_4 levels were measured. Ozone reduced serum T_4 levels in normal rats, but not the other groups of rats which had reduced levels of T_4 prior to exposure. However, when the thyroidectomized and hypophysectomized rats received supplemental T_4 in their drinking water that increased their serum T_4 level, O_3 caused a decrease in serum T_4. Generally, the higher the pre-exposure T_4 levels, the greater the O_3-induced reduction in T_4 levels. Similar observations were made for thyroidectomized rats when serum T_3 levels were measured. Exposure to O_3 also decreased plasma TSH levels in normal rats and in thyroidectomized rats supplemented with T_4 in the drinking water. These observations led the authors to hypothesize that the results are not due to a reduction in the hypothalamus-pituitary-thyroid axis as previously suggested (Clemons and Garcia, 1980a,b), but that O_3 causes decreases in serum T_3, T_4, and TSH by peripheral changes, possibly changes in serum binding.

The susceptibility of the parathyroid gland to O_3 exposure was investigated by Atwal and co-workers. In the initial study (Atwal and Wilson, 1974), rabbits were exposed to 1470 $\mu g/m^3$ (0.75 ppm) of O_3 for 4 to 8 hr, and the parathyroid gland was examined with light and electron microscopy at 6, 18, 22, and 66 hr after exposure. The parathyroid gland exhibited increased activity after O_3 exposure. Changes included hyperplasia of chief cells; hypertrophy and proliferation of the rough endoplasmic reticulum, free ribosomes, mitochondria, Golgi complex, and lipid bodies; and an increase of secretion granules within the vascular endothelium and capillary lumen. Such changes suggested an increased synthesis and release of parathormone, but actual hormone levels were not measured.

Atwal et al. (1975) also investigated possible autoimmune involvement in parathyroiditis of rabbits following a 48-hr exposure to 1470 μg/m^3 (0.75 ppm) of O_3. The authors stated that there was both a "continuous" and an "intermittent" 48-hr exposure, without specifying which results were due to which exposure regimen. Animals were examined between 1 and 20 days postexposure. Hyperplastic parathyroiditis was observed to be followed by capillary proliferation and leukocytic infiltration. Cytologic changes included the presence of eosinophilic leukocytes, reticuloendothelial and lymphocytic infiltration, disaggregation of the parenchyma, and interstitial edema. A variety of alterations were observed by electron microscopy, including atrophy of the endoplasmic reticulum of the chief cells, atrophy of mitochondria, degeneration of nuclei, and proliferation of the venous limb of the capillary bed. The alterations to the parathyroid gland were progressive during postexposure periods. Parathyroid-specific autoantibodies were detected in the serum of O_3-exposed rabbits, suggesting that the parathyroiditis might be due to inflammatory injury with an autoimmune causation. Microvascular changes in the parathyroid were further studied by Atwal and Pemsingh (1981) in dogs exposed to 1470 μg/m^3 (0.75 ppm) of O_3 for 48 hr. They reported focal hemorrhages, vascular endothelial proliferation, intravascular platelet aggregation, and lymphocytic infiltration. A potential autoimmunity after O_3 exposure was also observed by Scheel et al. (1959), who showed the presence of circulating antibodies against lung tissue.

In another study, Pemsingh and Atwal (1983) studied cells (APUD-type) within ciliated cysts of the parathyroid gland of dogs exposed for 48 hr to 1470 μg/m^3 (0.75 ppm) O_3. Examination was made at several times post-exposure (3, 10-13, and 28 days). Ozone caused ciliated cysts in 5 out of 12 exposed dogs and 1 out of 4 control dogs. Cysts in the O_3-exposed dogs had different cellular content, namely the presence of APUD-type cells on the cyst wall. The interpretation of these findings is not clear. The ultrastructure of chief cells, which are the hormone-producing cells, was also examined in dogs exposed identically to those described above (Atwal and Pemsingh, 1984). Mallory body-like inclusions (i.e., accumulations of filaments in the perinuclear area) were found within the chief cells of the parathyroid of O_3-exposed dogs. Additional alterations were observed. The highest incidence of this change was at 10 to 13 days post-exposure.

Also classed as a hormone is 5-hydroxytryptamine (5-HT), and it too interacts with O_3 toxicity (Suzuki, 1976). It has a variety of activities, including bronchoconstriction and increased capillary permeability; it can be a neurotransmitter. Although rats were exposed to a high concentration (9800 $\mu g/m^3$, 5.0 ppm) of O_3 for 3 hr, this study is discussed because extrapulmonary effects were observed. This exposure caused an increase in the 5-HT content of the lung and spleen, a decrease in 5-HT in the brain, and no change in the levels of 5-HT in the liver or kidney.

Adrenal cortex function after a 93-day (continuous) exposure to O_3 was investigated in rats (Eglite, 1968). Exposure to 110 $\mu g/m^3$ (0.056 ppm), but not lower levels, increased the urine concentration of 17-ketosteroids from the 6th week of exposure to the end of exposure. There was also a decrease in ascorbic acid in the adrenals. The generation and monitoring methods for the O_3 exposures were not sufficiently described. The authors described the effects as statistically significant but did not specify the statistical methods. No data were provided. Therefore, these results need to be confirmed before accurate interpretation is possible.

9.4.6.3 Other Effects. Rats were exposed for 6 hr/day, 4 days/week, for about 19 weeks, and analyses were performed on urine collected for the 16 hr following the exposure week (Hathaway and Terrill, 1962). Ozone exposures were uncontrolled, ranging from 1568 to 2940 (0.8 to 1.5 ppm). All parameters were not measured for each week of exposure. On days 91 and 112 after initial exposure, there was a lower titratable acidity and higher pH in the urine of O_3-exposed animals. Titratable acidity was also lower on day 98. Ozone did not alter the levels of creatinine, creatine, uric acid/ creatinine, amino acid nitrogen/creatinine excretions, or excretion of 12 amino acids. The lungs and kidneys were examined histologically, and no consistent differences were observed. The authors interpreted the results as a reflection of respiratory alkalosis, assuming no kidney toxicity.

Gastric secreto-motor activities of the rat were investigated by Roth and Tansy (1972). A 2-hr exposure to 490 $\mu g/m^3$ (0.25 ppm) caused no effects. Thirty minutes of exposure to 2940 $\mu g/m^3$ (1.5 ppm) inhibited gastric motility, but activity tended to return towards normal for the remaining 90 min of exposure. Recovery had occurred by 20 min postexposure. However, these results are questionable because ozone was monitored only by ozonator flow rates.

9.5 EFFECTS OF OTHER PHOTOCHEMICAL OXIDANTS

9.5.1 Peroxyacetyl Nitrate

Very little information on the toxicity of peroxyacetyl nitrate (PAN) has appeared in the literature since the previous criteria document on photochemical oxidants (U.S. Environmental Protection Agency, 1978). The document reviewed the results of inhalation experiments with mice that tested PAN's lethal concentration (LC_{50}) (Campbell et al., 1967); its effects on lung structure (Dungworth et al., 1969); and its influence on susceptibility to pulmonary bacterial infections (Thomas et al., 1979). The concentration of PAN used in these studies ranged from 22.3 mg/m^3 (4.5 ppm) to 750 mg/m^3 (150 ppm). They are considerably higher than the 0.232 mg/m^3 (0.047 ppm) daily maximum concentration of PAN reported in recent years for ambient air samples in areas having relatively high oxidant levels (Chapter 5) and are of questionable relevance to the assessment of effects on human health.

Campbell et al. (1967) estimated that the LC_{50} for mice ranged from 500 to 750 mg/m^3 (100 to 150 ppm) for a 2-hr exposure to PAN at 80°F (25°C). Mice in the 60- to 70-day-old age group were more susceptible to PAN lethality than mice ranging from 98 to 115 days in age. Temperature also influenced the lethal toxicity of PAN; a higher LC_{50} (less susceptibility) was seen at 70°F (125 ppm) than at 90°F (85 ppm). In a follow-up study, Campbell et al. (1970) characterized the behavioral effects of PAN by determining the depression of voluntary wheel-running activity in mice. Exposures to 13.9, 18.3, 27.2, 31.7, and 42.5 mg/m^3 (2.8, 3.7, 5.5, 6.4, and 8.6 ppm) of PAN for 6 hr depressed both the 6-hr and 24-hr activity when compared to similar pre-exposure periods. Depression was more complete and occurred more rapidly with higher exposure levels. However, the authors indicated that PAN was less toxic than O_3 when compared to similar behavioral data reported by Murphy et al. (1964) (Section 9.4.1).

Dungworth et al. (1969) reported that daily exposures of mice to 75 mg/m^3 (15 ppm) of PAN 6 hr/day for 130 days caused a 30-percent weight loss compared to sham controls, 18 percent mortality, and pulmonary lesions. The most prevalent lesions were chronic hyperplastic bronchitis and proliferative peribronchiolitis.

Thomas et al. (1979) found that mice exposed to 22.3 mg/m^3 of PAN (4.5 ppm) for 2 hr and subsequently challenged with a Streptococcus sp. aerosol for 1 hr showed a significant increase in mortality and a reduction in mean survival rate, compared to mice exposed to air. No effect on the incidence of fatal pulmonary infection or survival time was observed in mice challenged with Streptococcus sp. 1 hr before the pollutant exposure (27.2 mg/m^3 of PAN for 3 hr). Thomas et al. (1981a) published additional data that extend observations of reduced resistance of mice to streptococcal pneumonia over a range of exposures to PAN. A single 2- or 3-hr exposure to PAN at 14.8 to 28.4 mg/m^3 (3.0 to 5.7 ppm) caused a significant increase in the susceptibility of mice to streptococcal pneumonia. The mean excess mortality rate ranged from 8 to 39 percent. Mice exposed to O_3 at 0.98 mg/m^3 (0.5 ppm) and challenged with the Streptoccus sp. aerosol resulted in a mean excess mortality (38 percent) that was almost equivalent to the excess mortality for the group exposed to 28.4 mg/m^3 of PAN. The results agreed with earlier reports that PAN is less toxic than O_3 to mice exposed under ambient conditions. Exposure to 7.4 mg/m^3 (1.5 ppm) of PAN 3 hr/day, 5 days/week for 2 weeks had no appreciable effect, although no statistics were provided. Neither exposure routine altered the morphology, viability, or phagocytic activity of isolated macrophages, although there was a decrease in ATP levels. The other noticeable effect was that macrophages isolated from the animals that were repeatedly exposed failed to attach themselves to a glass substrate. These investigators also studied whether a chronic infection initiated with an exposure to Mycobacterium tuberculosis (RIRv) was influenced by subsequent exposure to PAN. The exposure to this oxidant (25 mg/m^3 for 6 days) did not alter the pattern of bacterial growth in the lungs of mice.

9.5.2 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) has been reported to occur in trace amounts in urban air samples (Chapter 5), but very little is known about the effects of H_2O_2 from inhalation exposure. Most of the early work on H_2O_2 toxicity involved exposure to very high concentrations. Oberst et al. (1954) investigated the inhalation toxicity of 90 percent H_2O_2 vapor in rats, dogs, and rabbits at concentrations ranging from 10 mg/m^3 (7 ppm) daily for six months to an 8-hr exposure to 338 mg/m^3 (243 ppm). After autopsy, all animals showed abnormalities of the lung. In a recent experiment, Last et al. (1982) exposed rats for 7 days to > 95-percent H_2O_2 gas with a concentration of 0.71 mg/m^3 (0.5 ppm) in the presence of respirable ammonium sulfate particles. No significant effects were observed in body weight, lung lobe weights, and protein or DNA content of lung homogenates. The authors suggested that because H_2O_2 is highly soluble, it is not expected to penetrate to the deep lung, which may account for the absence of observed effects.

The majority of studies on H_2O_2 explore possible mechanisms for the effects of H_2O_2. These include direct cellular effects associated with *in vitro* exposure to H_2O_2 and biochemical reactions to H_2O_2 generated *in vivo*.

Hydrogen peroxide may affect lung function by the alteration of pulmonary surfactant. Wilkins and Fettissoff (1981) found that 10^{-2} to 10^{-1}M of H_2O_2 increased the surface tension of saline extracts of dog lung homogenates. The authors estimated that a dog breathing 1.4 mg/m^3 (1 ppm) of H_2O_2 for 30 hr would build up a pollutant concentration of 10^{-2}M in the surfactant, assuming that all the H_2O_2 was retained by the lungs. However, as stated above, this estimate of tissue dose is not realistic, because most of the H_2O_2 would be absorbed in the upper airway.

Another mechanism by which H_2O_2 may affect ventilation is by changing the tone of airway smooth muscle. Stewart et al. (1981) reported that 10^{-4}M of H_2O_2 caused significant constriction of strips of subpleural canine lung parenchyma and of bovine trachealis muscle. In the distal airway preparation (canine), this contraction was reversed by catalase. Pretreatment of both proximal (bovine) and distal muscle strips with meclofenamate or iodomethacin markedly reduced the response to H_2O_2. This suggested that the increase in airway smooth muscle tone produced by H_2O_2 involves prostaglandin-like substances.

The potential genotoxic effects from *in vitro* H_2O_2 exposure have been evaluated in isolated cell systems. Bradley et al. (1979) reported that H_2O_2 produced both toxicity and single-strand DNA breaks but was not mutagenic at concentrations up to 530 μM. They observed a significant increase in the frequency of reciprocal sister chromatid exchanges in V-79 Chinese hamster cells at a concentration of 353 μM of H_2O_2. However, the authors pointed out that sister chromatid exchange frequency was not necessarily equivalent to increased mutant frequency. In subsequent experiments, Bradley and Erickson (1981) confirmed these observations in V-79 Chinese hamster lung cells and were unable to detect any DNA-protein or DNA-DNA crosslinks with 353 μM of H_2O_2 for 3 hr at 37°C. Increases in the frequency of sister chromatid exchanges in Chinese hamster cells have also been found by MacRae and Stich (1979), Speit and Vogel (1982), and Speit et al. (1982). Wilmer and Natarajan (1981) reported only a slight enhancement in the frequency of sister chromatid exchanges in Chinese hamster ovary cells following treatment with up to 10^{-3}M of H_2O_2. In comparison, cells were killed with a concentration of 10^{-1}M of H_2O_2. Similarly, H_2O_2 (10^{-1}mg/ml) was negative in the Ames mutagenicity assay (Ishidate and Yoshikawa, 1980), and several other investigators have confirmed the lack of mutagenicity for H_2O_2 (Stich et al., 1978; Kawachi et al., 1980).

Johnson et al. (1981) reported that the intrapulmonary instillation of glucose oxidase, a generator of H_2O_2, increases lung permeability in rats. A greater increase in lung permeability was achieved by the addition of a combination of glucose oxidase and lactoperoxidase than by the glucose oxidase-H_2O_2-generating system alone. Horseradish peroxidase did not effectively substitute for lactoperoxidase in the potentiation of damage. Injury was blocked by catalase but not by superoxide dismutase (SOD), suggesting that H_2O_2 or its metabolites, rather than superoxide, were involved. Because horseradish peroxidase did not potentiate the glucose oxidase damage, the authors speculated that the mechanism of injury occurs through the action of a halide-dependent pathway described for cell injury produced by H_2O_2 and lactoperoxidase/myeloperoxidase (MPO) (Klebanoff and Clark, 1975). Any source of H_2O_2 plus MPO and a halide cofactor is capable of catalyzing many oxidation and halogenation reactions (Clark and Klebanoff, 1975), but other possible mechanisms of oxygen radical production have been proposed (Halliwell, 1982).

Carp and Janoff (1980) have shown that a H_2O_2 generating system with MPO and Cl^- will suppress the elastase inhibitory capacity of the protease inhibitor (BMPL) present in bronchial mucus. This antiprotease is capable of inhibiting the potentially dangerous proteases found in human polymorphonuclear leukocytes (PMNs), including elastase and cathepsin-G. Inactivation of BMPL could make the respiratory mucosa more susceptible to attack by inflammatory cell proteases. Human PMN have not been shown to contain MPO but macrophages may contain analogous forms of peroxidase.

In other in vitro experiments, Suttorp and Simon (1982) demonstrated that H_2O_2 generated by glucose oxidase was cytotoxic to cultured lung epithelial cells (L_2 cells) in a concentration-dependent fashion. Cytotoxicity was measured by determining ^{51}Cr release from target cells. Cytotoxicity was prevented by the addition of catalase. It was stressed that there is no established identity between the L_2 cell line and the in situ type 2 pneumocytes from which they were derived.

9.5.3 Formic Acid

Toxicological interest in formic acid has focused primarily on its role as the metabolic end product of methanol and on any similarity the effects from inhalation exposure to formic acid vapor may have with methanol toxicity. Unfortunately, the concentrations (20 ppm) used in the two studies discussed below are over three orders of magnitude greater than the trace concentrations ($\leq$0.015 ppm) reported in the highest oxidant areas of Southern California (see Chapter 5).

Zitting and Savolainen (1980) exposed rats to 20 ppm (0.8 µmol/L) formic acid vapor, 6 hr/day for 3 and 8 days. Tissue samples were analyzed for neurochemical effects as well as effects on drug-metabolizing enzymes in the liver and kidneys. Enzyme profiles were variable, depending on the sampling period during exposure. However, a general pattern developed. Cerebral glutathione concentration and acid proteinase activity increased after 3 and 8 days of exposure, respectively, indicating possible lipid peroxidation associated with cerebral hypoxia. Decreased kidney ethoxycoumarin deethylase activity, cytochrome P-450 content, and glutathione concentrations were also consistent with changes due to lipid peroxidation. The liver, which is less sensitive to tissue hypoxia, showed only small increases in deethylase activity although associated with a reduction in glutathione. Since prolonged low-level cerebral hypoxia can potentially lead to demyelination and subsequent nerve degeneration, the authors repeated the study to look specifically at effects on glial cells (Savolainen and Zitting, 1980). Rats were exposed to 20 ppm formic acid vapor, 6 hr/day, 5 days/week for 2 or 3 weeks. Again, enzyme profiles were indicative of metabolic responses to tissue hypoxia, providing evidence for potential central nervous system toxicity at high formic acid vapor concentrations.

9.5.4 Complex Pollutant Mixtures

Additional toxicological studies have been conducted on the potential action of complex mixtures of oxidants and other pollutants. Animals have been exposed under laboratory conditions to ambient air from high oxidant areas, to UV-irradiated and nonirradiated reaction mixtures of automobile exhaust and air, and to other combinations of interactive pollutants. Although these mixtures attempt to simulate the photochemical reactions produced under actual atmospheric conditions, they are extremely difficult to analyze because of their chemical complexity. Variable concentrations of total oxidants, carbon monoxide, hydrocarbons, nitrogen oxides, sulfur oxides, and other unidentified complex pollutants have been reported. For this reason, the studies presented in this section differ from the more simplified combinations of O_3 and one or two other nonreactive pollutants discussed under previous sections of the chapter. The effects described in animals exposed to UV-irradiated exhaust mixtures are not necessarily uniquely characteristic of O_3, but most of them could have been produced by O_3. In most cases, however, the biological effects presented would be difficult to associate with any one pollutant.

Research on ambient air and UV-irradiated or nonirradiated exhaust mixtures is summarized in Table 9-27. Long-term exposure of various species of animals to ambient California atmospheres have produced changes in the pulmonary function of guinea pigs (Swann and Balchum, 1966; Wayne and Chambers, 1968) and have produced a number of biochemical, pathological, and behavioral effects in mice, rats, and rabbits (Wayne and Chambers, 1968; Emik and Plata, 1969; Emik et al., 1971). Exposure to UV-irradiated automobile exhaust containing oxidant levels of 0.2 to 1.0 ppm produced histopathologic changes (Nakajima et al., 1972) and increased susceptibility to infection (Hueter et al., 1966) in mice. Both UV-irradiated and nonirradiated mixtures produced decreased spontaneous running activity (Hueter et al., 1966; Boche and Quilligan, 1960) and decreased infant survival rate and fertility (Kotin and Thomas, 1957; Hueter et al., 1966; Lewis, et al., 1967) in a number of experimental animals. Pulmonary changes were demonstrated in guinea pigs after short-term exposure to irradiated automobile exhaust (Murphy et al., 1963; Murphy, 1964) and in dogs after long-term exposure to both irradiated and nonirradiated automobile exhaust (Lewis et al., 1974; Orthoefer et al., 1976). Irradiation of the air-exhaust mixtures led to the formation of photochemical reaction products that were biologically more active than nonirradiated mixtures. The concentration of total oxidant as expressed by O_3 ranged from 588 to 1568 $\mu g/m^3$ (0.30 to 0.80 ppm) in the irradiated exhaust mixtures, compared to only a trace or no oxidant detected in the nonirradiated mixtures.

The description of effects following exposure of dogs for 68 months to automobile exhaust, simulated smog, oxides of nitrogen, oxides of sulfur, and their combination has been expanded in a monograph by Stara et al. (1980). The dogs were examined after 18 months (Vaughan et al., 1969), 36 months

TABLE 9-27. EFFECTS OF COMPLEX POLLUTANT MIXTURES

Concentration,[a] (ppm)	Pollutant[b]	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
			A. Ambient air		
0.032 - 0.050 9.1 - 13.5 0.044 - 0.077 0.019 - 0.144	O_x CO NO_2 NO	Lifetime study, continuous	No clear chronic effects; "suggestive" changes in pulmonary function, morphology, and incidence of pulmonary adenomas in aged animals. Increased 17-ketosteroid excretion in guinea pigs. Decreased glutamic oxalacetic transaminase in blood serum of rabbits.	Mouse, rat, hamster, guinea pig, rabbit	Wayne and Chambers, 1968
0.057 1.7 2.4 0.019 0.015 0.004	O_x CO HC NO_2 NO PAN	2.5 years, continuous	Reduced pulmonary alkaline phosphatase (rats); reduced serum glutamic oxaloacetic transaminase (rabbits); increased pneumonitis (mice); increased mortality (male mice); reduced body weights (mice); decreased running activity (male mice); no significant induction of lung adenomas (mice).	Mouse, rat, rabbit	Emik et al., 1971
0.062 - 0.239 0.03 - 0.07 2.7 - 4.4	O_x NO_2 HC	13 months, continuous	Decreased spontaneous running activity.	Mouse	Emik and Plata, 1969.
0.27 - 0.31 13 - 38 2 - 9 0.09 - 0.70	O_x CO HC NO_x	1 year, continuous	Expiratory flow resistance increased on days when O_x reached $\geq$ 0.30 ppm or at combined concentrations of $\geq$ 40 ppm of CO, 16 ppm of HC, and 1.2 ppm of NO_x. Individual sensitivity demonstrated. Temperature was an important variable.	Guinea pig	Swann and Balchum, 1966
0.4 (max)	O_x	19 weeks, continuous	No consistent effects on conception rate, litter rate, or newborn survival.	Mouse	Kotin and Thomas, 1957

TABLE 9-27. EFFECTS OF COMPLEX POLLUTANT MIXTURES (continued)

Concentration,[a] (ppm)	Pollutant[b]	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
			B. Automobile exhaust		
0.012- 0.65 3.0 0.04 - 1.10 0.06 - 5.00 0.06 - 1.20	O_3 (max) HC (propylene) NO_2 NO SO_2	0.5-6 hr (diesel)	UV-irradiation of propylene, SO_2, NO, and NO_2 produced O_3 and a mutagenic moiety when collected particles were tested by the plate-incorporation test. Irradiation did not alter and O_3 tended to reduce the mutagenic response.	Salmonella typhimurium	Claxton and Barnes, 1981
0.04 - 0.2 0.15 - 0.5 0.4 - 1.8 6 - 36 20 - 100	O_3 NO_2 NO HC (CH_4) CO	1.5 - 23 mo	Increased pulmonary infection. Decreased fertility and infant survival. No significant changes in pulmonary function. Decreased spontaneous running activity during the first few weeks of exposure.	Mouse, rat, hamster, guinea pig	Hueter et al., 1966 Lewis et al., 1967
0.1 - 0.5 0.2 - 0.6 5 - 8 40 - 60	O_x NO_x HC CO	4 weeks, 5 days/week, 2-3 hr/day	Histopathologic changes resembling tracheitis and bronchial pneumonia at the higher concentration range of oxidants.	Mouse	Nakajima et al., 1972
0.2 - 0.4 100 24 - 30 0.1 - 1.0 0.1 - 2.0 0.42 - 0.49 0.02 - 0.03	O_3 CO HC (CH_4) NO_2 NO SO_2 H_2SO_4	18-68 months, 7 days/week, 16 hr/day	No cardiovascular effects	Dog	Bloch et al., 1972, 1973 Gillespie, 1980
		2-3 years recovery in ambient air.	No significant differences in collagen: protein ratios; prolyl hydroxylase increased with high concentrations of all mixes.	Dog	Orthoefer et al., 1976
			Pulmonary function for groups receiving oxidants [irradiated exhaust (I) ± SO_x] 18 months: no effects 36 months: no effects	Dog	Vaughan et al., 1969 Lewis et al., 1974

TABLE 9-27. EFFECTS OF COMPLEX POLLUTANT MIXTURES (continued)

Concentration,[a] (ppm)	Pollutant[b]	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
			61 months: N_2 washout increased (I); R_L increased (I, I+SO_x).	Dog	Lewis et al., 1974
			2 years recovery: P_aCO_2 increased (I+SO_x); V_D increased (I, I+SO_x); D_{Lco}/TLC decreased and V_c increased in all groups; lung compartment volumes increased (I+SO_x).	Dog	Gillespie, 1980
			Morphology (32-36 months recovery): air space enlargement; nonciliated bronchiolar hyperplasia; foci of ciliary loss with and without squamous metaplasia in trachea and bronchi.	Dog	Hyde et al., 1978
0.33 - 0.82 0.16 - 5.50 0.16 - 4.27 0.12 - 2.42 0.02 - 0.20	O_x NO_2 NO Formaldehyde Acrolein	4-6 hr	Increased pulmonary flow resistance, increased tidal volume, decreased breathing frequency due to formaldehyde and acrolein at low O_x: aldehyde ratio. Decreased tidal volume, increased frequency, increased pulmonary resistance due to O_x and NO_x at high O_x: aldehyde ratio.	Guinea pig	Murphy et al., 1963; Murphy, 1964
C. Other complex mixtures					
0.08 0.76 2.05 1.71	O_3 SO_2 T-2 Butene acetaldehyde	4 weeks 7 days/week 23 hr/day	Alteration in distribution of ventilation (ΔN_2) and increased diffusing capacity.	Hamster	Raub et al., 1983b
0.3 1.0 2.0	O_3 NO_2 SO_2	2 weeks 7 days/week 23 hr/day	Voluntary activity (wheel running) decreased 75% after 1-3 days, returning to 85% of pre-exposure levels by the end of 14 days.	Mouse	Stinson and Loosli, 1979

TABLE 9-27. EFFECTS OF COMPLEX POLLUTANT MIXTURES (continued)

Concentration,[a] (ppm)	Pollutant[b]	Exposure duration and protocol	Observed effect(s)[c]	Species	Reference
0.40 - 0.52 1.0 - 2.15	O_3 O_x (gas vapor)	24 hr	Decreased spontaneous wheel running activity.	Mouse	Boche and Quilligan, 1960
1.25	Ox (gas vapor)	19 weeks, continuous	Decreased conception rate, litter rate, and newborn survival.	Mouse	Kotin and Thomas, 1957

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[a] Ranked by nonspecific oxidant concentration (O_3 or O_x).

[b] Abbreviations used: O_3 = ozone; O_x = oxidant; CO = carbon monoxide; NO = nitrogen oxide, NO_2 = nitrogen dioxide; NO_x = nitrogen oxides; SO_2 = sulfur dioxide; HC = hydrocarbon; CH_4 = methane; H_2SO_4 = sulfuric acid; PAN = peroxyacetyl nitrate.

[c] See Glossary for the identification of pulmonary symbols.

(Lewis et. al., 1974), 48 to 61 months (Bloch et al., 1972, 1973; Lewis et al., 1974), and 68 months (Orthoefer et al., 1976) of exposure; the dogs were examined again 24 months (Gillespie, 1980) or 32 to 36 months (Orthoefer et al., 1976; Hyde et al., 1978) after exposure ceased. Only those results pertaining to oxidant exposure are described in this section, which limits the discussion to groups exposed to irradiated automobile exhaust (I) and irradiated exhaust supplemented with sulfur oxides ($I+SO_x$). See Table 9-27 for exposure concentrations.

No specific cardiovascular effects were reported during the course of exposures (Bloch et al., 1971, 1972, 1973) or 3 years after exposure (Gillespie, 1980). Similarly, Orthoefer et al. (1976) reported no significant biochemical differences in the collagen to protein ratio in tissues of dogs exposed for 68 months or after 2.5 to 3 years of recovery in ambient air. However, prolyl hydroxylase levels were reported to have increased in the lungs of dogs exposed to I and $I+SO_x$, when compared to control air and the nonirradiated exhaust alone or in combination with SO_x.

No significant impairment of pulmonary function was found after 18 months (Vaughan et al., 1969) or 36 months (Lewis et al., (1974) of exposure. However, by 61 months of exposure, Lewis et al. (1974) reported increases in the nitrogen washout of dogs exposed to I, and higher total expiratory resistance in dogs exposed to both I and $I+SO_x$, when compared to their respective controls receiving clean air and SO_x alone. Two years after exposure ceased, pulmonary function was remeasured by Gillespie (1980). These measurements were made in a different laboratory than the one used during exposure, but consistency among measurements of the control group and another set of dogs of similar age at the new laboratory indicated that this difference did not have a major impact on the findings. Arterial partial pressure of CO_2 increased in the group exposed to $I+SO_x$ and total deadspace increased in the the I and $I+SO_x$ groups. The diffusing capacity for carbon monoxide (D_{Lco}) was similar in all exposure groups, but when normalized for total lung capacity (TLC), the D_{Lco}/TLC ratio was smaller in exposed groups than in the air control group. Mean capillary blood volumes also increased in all exposed groups. No changes in lung volumes were reported at the end of exposure (Lewis et al., 1974). However, when lung volumes from these animals were measured 2 years later, increases were reported in the $I+SO_x$ group. Unfortunately, the sample size of dogs exposed to I was too small (n = 5) to permit meaningful comparisons. In general, pulmonary function changes were found to be similar in all groups exposed to automobile exhaust alone or supplemented with SO_x. Exposure to these mixtures with or without UV-irradiation produced lung alterations normally associated with injury to the airway and parenchyma.

The functional abnormalities mentioned above showed relatively good correlation with structural changes reported by Hyde et al. (1978). After 32 to 36 months of recovery in clean air, morphologic examination of the lungs by light microscopy, scanning electron microscopy, and transmission electron microscopy revealed a number of exposure-related effects. The displaced volume of the fixed right lung was larger in the I-exposed group. Both the I and I+SO_x groups showed random enlargement of alveolar airspaces centered in respiratory bronchioles and alveolar ducts. Small hyperplastic lesions were observed at the junction of the terminal bronchiole and the first-order respiratory bronchiole. Foci of ciliary loss associated with squamous metaplasia were also observed in the intrapulmonary bronchi of the I+SO_x group. However, because these was no significant difference in the magnitude of these lesions, oxidant gases and SO_x did not appear to act in an additive or synergistic manner.

Additional work on irradiated and nonirradiated automobile exhaust has been presented by Claxton and Barnes (1981). The mutagenicity of diesel exhaust particle extracts collected under smog-chamber conditions was evaluated by the Salmonella typhimurium plate-incorporation test (Ames et al., 1975). The authors demonstrated that the irradiation of propylene, SO_2, NO, and NO_2 produced O_3 and a mutagenic moiety. In baseline studies on diesel exhaust, in which O_3 was neither added nor produced, the mutagenicity of each sample was similar under dark or UV-light conditions. When O_3 was introduced into the smog chamber, the mutagenicity of the organic compounds was reduced.

The behavioral effect of a nonirradiated reaction mixture was examined by Stinson and Loosli (1979). Voluntary wheel-running activity was recorded during a continuous 2-week exposure to synthetic smog containing 588 $\mu g/m^3$ (0.3 ppm) of O_3, 1 ppm of NO_2, and 2 ppm of SO_2, or to each component separately. An immediate decrease in spontaneous activity occurred after 1 to 3 days of exposure, returning to 85 percent of the original activity by the end of exposure. Activity returned to basal levels 5 days after breathing filtered air. Ozone alone produced a response that was similar to that of the synthetic smog mixture. Since NO_2 and SO_2 alone had only moderate effects, the authors concluded that O_3 had the major influence on depression of activity.

More recently, Raub et al. (1983b) reported pulmonary function changes in hamsters exposed 23 hr/day for 4 weeks to a nonirradiated reaction mixture of trans-2-butene, O_3, and SO_2. Decreases in the nitrogen washout slope and increases in the diffusing capacity indicated a significant compensatory change in distribution of ventilation in the lungs of exposed animals. Animals compromised by the presence of elastase-induced emphysema were unable to respond to this pulmonary insult in the same manner as animals without impaired lung function.

9.6 SUMMARY

9.6.1 Introduction

The biological effects of O_3 have been studied extensively in animals and a wide array of toxic effects have been ascribed to O_3 inhalation. Although much has been accomplished to improve the existing data base, refine the concentration-response relationships and interpret better the mechanisms of O_3 effects, many of the present data were not accumulated with the idea that quantitative comparisons to man would be drawn. In many cases, only qualitative comparisons can be made. To maximize the extent that animal toxicological data can be used to estimate the human health risk of exposure to O_3, the qualitative as well as quantitative similarities between the toxicity of O_3 to animals and man must be considered more carefully in the future. Significant advances have been made in understanding the toxicity of O_3 through appropriate animal models. This summary highlights the significant results of selected studies that will provide useful data for better predicting and assessing, in a scientifically sound manner, the possible human responses to O_3.

Summary figures and tables are presented in the following sections. The practical purpose of this presentation of the data is to help the reader focus on what types of effects or responses have been reported, what concentrations have been tested (1.0 ppm and lower), and as a convenient list of references with each of the biological parameters measured. Studies were selected for inclusion in these figures and tables on the basis of specific criteria presented below:

1. Studies have been cited when the reported effects are clearly due to O_3 exposure. Effects due to mixtures of O_3 with other pollutants have been summarized in a separate figure and table. Studies involving exercise,

diet deficiencies, or other possible modifiers of response to O_3 have not been included.

2. Cited studies report the effects of O_3 exposure over a broad range of animal species and strains and for varying lengths of time. Specific details on animal species, exposure duration, and observed biological effects can be obtained from the tables within the body of this chapter.

3. Each closed symbol on the figures represents one or more studies conducted at that particular concentration that caused effects. Specific references can be found in the accompanying tables.

4. Each open circle represents one or more studies that used the given concentration, but reported no significant effects. No-effect levels are also indicated by brackets in the accompanying tables.

5. Only pulmonary function effects were divided by short-term (<14 days) and long-term exposures to follow the discussion in the text.

In order to keep this section brief and concise, it was necessary to be somewhat selective in determining what and how this information would be presented. A number of important factors, such as the specific length of exposure, were not included. Also, the parameter selected to illustrate a specific response was usually broad and very general. For example, the category "decreases in macrophage function" includes such diverse endpoints as measurements of lysosomal and phagocytic activity, macrophage mobility, or chemotactic response. These responses may or may not be related to one another. Thus, care must be taken in how these data are used and interpreted. The only appropriate use is to gain an overview of the broad array of the effects of ozone and the concentrations which did and did not cause these effects.

9.6.2 Regional Dosimetry in the Respiratory Tract

The amount of O_3 acting at a given site in the lung is related to the airway luminal concentration at that level. As a result, O_3 does not immediately interact with cellular components of the respiratory tract. Instead, it first comes into contact with the mucous or surfactant layer lining the airway. It should be noted that O_3 is quite reactive chemically. Reactions with components of this layer cause an increase in total absorption of O_3 in the upper airways and in a reduction of the amount of O_3 reaching sensitive tissues. The site at which uptake and subsequent interaction occur and the local dose (quantity of O_3 absorbed per unit area per time), along with cellular sensitivity, will determine the type and extent of the injury. Also, the capacity for responding to a specific dose may vary between animals and humans because of dissimilarities in detoxification systems, pharmacokinetics, metabolic rates, genetic makeup, or other factors. Thus, along with the above, a knowledge of the complex process of gas transport and absorption is crucial to understanding the effects of O_3 and other oxidants in humans.

The animal studies that have been conducted on ozone absorption are beginning to indicate the quantity and site of O_3 uptake in the respiratory tract. Experiments on the nasopharyngeal removal of O_3 in animals suggest that the fraction of O_3 uptake depends inversely on flow rate, that uptake is greater for nose than for mouth breathing, and that tracheal and chamber concentrations are positively correlated. Only one experiment measured O_3 uptake in the lower respiratory tract, finding 80 to 87 percent uptake by the lower respiratory tract of dogs (Yokoyama and Frank, 1972). At present, however, there are no reported results for human nasopharyngeal or lower respiratory tract absorption. Caution must be used in estimating nasopharyngeal uptake for normal respiration based upon experiments employing unidirectional flows.

To further an understanding of O_3 absorption, mathematical models have been developed to simulate the processes involved and to predict O_3 uptake by various regions and sites within the respiratory tract. The model of Aharonson et al. (1974) has been used to analyze nasopharyngeal uptake data. Applied to O_3 data, the model indicates that the average mass transfer coefficient in the nasopharyngeal region increases with increasing air flow, but the actual percent uptake decreases.

Three models have been developed to simulate lower respiratory uptake (McJilton et al., 1972; Miller et al., 1978b, 1985). These models are very similar in their treatment of O_3 in the airways (taking into account convection, diffusion, wall losses, and ventilatory patterns) and in their use of morphological data to define the dimensions of the airways and liquid lining. The models differ in their treatment of the mechanism of absorption. Both of the models of Miller and co-workers take into account chemical reactions of O_3 with constituents of the liquid lining, whereas the model of McJilton et al.

does not. The models of Miller et al. differ in their treatment of chemical reactions, as well as in the fact that the newer model includes chemical reactions of O_3 in additional compartments, such as tissue and blood.

Tissue dose is predicted by the models of Miller et al. to be relatively low in the trachea, to increase to a maximum between the junction of the conducting airways and the gas-exchange region, and then to decrease distally. This is not only true for animal simulations (guinea pig and rabbit) but it is also characteristic of the human simulations (Miller et al., 1978b; 1985).

A comparison of the results of Miller and co-workers with morphological data (that shows the centriacinar region to be most affected by O_3) indicates qualitative agreement between predicted tissue doses and observed effects in the pulmonary region. However, comparisons in the tracheobronchial region indicate that dose-effect correlations may be improved by considering other expressions of dose such as total absorption by an airway and by further partitioning of the mucous layer compartment in mathematical models. Further research is needed to define toxic mechanisms, as well as to refine our knowledge of important chemical, physical, and morphological parameters.

At present, there are few experimental results that are useful in judging the validity of the modeling efforts. Such results are needed, not only to understand better the absorption of O_3 and its role in toxicity, but also to support and to lend confidence to the modeling efforts. With experimental confirmation, models which further our understanding of the role of O_3 in the respiratory tract will become practical tools.

The consistency and similarity of the human and animal lower respiratory tract dose curves obtained thus far lend strong support to the feasibility of extrapolating to man the results obtained on animals exposed to O_3. In the past, extrapolations have usually been qualitative in nature. With additional research in areas which are basic to the formulation of dosimetry models, quantitative dosimetric differences among species can be determined. If in addition, more information is obtained on species sensitivity to a given dose, significant advances can be made in quantitative extrapolations and in making inferences about the likelihood of effects of O_3 in man. Since animal studies are the only available approach for investigating the full array of potential disease states induced by exposure to O_3, quantitative use of animal data is in the interest of better establishing O_3 levels to which man can safely be exposed.

9.6.3 Effects of Ozone on the Respiratory Tract

9.6.3.1 Morphological Effects. The morphological changes which follow exposure to less than 1960 $\mu g/m^3$ (1.0 ppm) O_3 are very similar in all species of laboratory mammals studied. Of the many specific cell types found in the respiratory system, two types, ciliated cells and type 1 alveolar epithelial cells, are the cells most damaged morphologically following O_3 inhalation. Ciliated cells are found in the conducting airways, e.g., trachea, bronchi, and nonrespiratory bronchioles. Ciliated cells function in the normal clearance of the airways and the removal of inhaled foreign material. Following O_3 exposure of experimental animals, damaged ciliated cells have been reported in all of these conducting airways (Schwartz et al., 1976; Castleman et al., 1977). In rats, damage to ciliated cells appears most severe at the junction of the conducting airways with the gas exchange area (Stephens et al., 1974a; Schwartz et al., 1976). Damage to type 1 alveolar epithelial cells is limited to those cells located near this junction, i.e., the centriacinar or proximal alveolar region of the pulmonary acinus (Stephens et al., 1974b; Schwartz et al., 1976; Castleman et al., 1980; Barry et al., 1983; Crapo et al., 1984). Type 1 alveolar cells form most of the blood-air barrier where gas exchange occurs. Severely damaged ciliated and type 1 alveolar epithelial cells are shed (sloughed) from the tissue surface and are replaced by multiplication of other cell types less damaged by O_3 (Evans et al., 1985). This process has been most extensively studied in the centriacinar region where nonciliated bronchiolar cells and type 2 alveolar epithelial cells become more numerous (Evans et al., 1976a,b,c; Lum et al., 1978). Some of these nonciliated bronchiolar and type 2 cells differentiate into ciliated and type 1 cells, respectively. Cell multiplication in bronchioles may be more than that required for replacement of damaged ciliated cells, and nonciliated bronchiolar cells may become hyperplastic (Castleman et al., 1977; Ibrahim et al., 1980; Eustis et al., 1981) and sometimes appear as nodules (Zitnik et al., 1978; Moore and Schwartz, 1981; Fujinaka et al., 1985). Inflammatory changes characterized by a variety of leukocytes with alveolar macrophages predominating, intramural edema, and fibrin are also seen in the centriacinar region (Stephens et al., 1974a; Schwartz et al., 1976; Castleman et al., 1977; Fujinaka et al., 1985).

The damage to ciliated and centriacinar type 1 alveolar epithelial cells and the inflammatory changes tend to occur soon after exposure to concentrations of O_3 as low as 392 μg/m^3 (0.2 ppm). Damage to centriacinar type 1 alveolar epithelium in rats has been well documented as early as 2 hours after exposure to O_3 concentrations of 980 μg/m^3 (0.5 ppm) (Stephens et al., 1974a). In the same publication the authors report damage to centriacinar type 1 alveolar epithelial cells after 2 hours exposure to 392 μg/m^3 (0.2 ppm) O_3, but this portion of their report is not documented by published micrographs (Stephens et al., 1974a). Loss of cilia from cells in the rat terminal bronchiole occurs following exposure to 980 μg/m^3 (0.5 ppm) O_3 for 2 hours (Stephens et al., 1974a). Damage to ciliated cells has been seen following exposure of both rats and monkeys to 392 μg/m^3 (0.2 ppm) O_3, 8 hr/day for 7 days (Schwartz et al., 1976; Castleman et al., 1977). Centriacinar inflammation has been reported as early as 6 hours after exposure to 980 μg/m^3 (0.5 ppm) O_3 (Stephens et al., 1974b) and 4 hours after exposure to 1568 μg/m^3 (0.8 ppm) O_3 (Castleman et al., 1980).

During long-term exposures, the damage to ciliated cells and to centriacinar type 1 cells and centriacinar inflammation continue, though at a reduced rate. Damage to cilia has been reported in monkeys following 90-day exposure to 980 μg/m^3 (0.5 ppm) O_3, 8 hr/day (Eustis et al., 1981) and in rats exposed to 980 μg/m^3 (0.5 ppm) O_3, 24 hr/day for 180 days (Moore and Schwartz, 1981). Damage to centriacinar type 1 cells was reported following exposure of young rats to 490 μg/m^3 (0.25 ppm) O_3, 12 hrs/day for 42 days (Barry et al., 1983; Crapo et al., 1984). Changes in type 1 cells were not detectable after 392 μg/m^3 (0.2 ppm) O_3, 8 hr/day for 90 days but were seen in rats exposed to 980 μg/m^3 (0.5 ppm) for the same period (Boorman et al., 1980). Centriacinar inflammatory changes persist during 180-day exposures of rats to 980 μg/m^3 (0.5 ppm) O_3, 24 hr/day (Moore and Schwartz, 1981) and one-year exposures of monkeys to 1254 μg/m^3 (0.64 ppm) O_3, 8 hr/day (Fujinaka et al., 1985).

Remodeling of distal airways and centriacinar regions occurs following long-term exposures to O_3. Rats develop respiratory bronchioles between the terminal bronchiole to alveolar duct junction seen in control rats (Boorman et al., 1980; Moore and Schwartz, 1981). In monkeys, distal airway remodeling results in increased volumes of respiratory bronchioles which have thicker walls and a smaller internal diameter (Fujinaka et al., 1985). The walls of centriacinar alveoli are also thickened (Schwartz et al., 1976; Boorman et al., 1980; Barry et al., 1983; Crapo et al., 1984; Last et al., 1984a). Studies of the nature of these thickened interalveolar septa and bronchiolar walls revealed increases in inflammatory cells, fibroblasts, and amorphous extracellular matrix (Last et al., 1984a; Fujinaka et al., 1985). Three studies provide morphological evidence of mild fibrosis (i.e., local increase of collagen) in centriacinar interalveolar septa following exposure to < 1960 $\mu g/m^3$ (< 1 ppm) of O_3 (Last et al., 1979; Boorman et al., 1980; Moore and Schwartz, 1981). Changes in collagen location or amounts, or both, which occur with the remodeling of the distal airways, were reported in two of those studies (Boorman et al., 1980; Moore and Schwartz, 1981).

While morphometry of small pulmonary arteries is not commonly studied in O_3-exposed animals, pulmonary artery walls thickened by muscular hyperplasia and edema were reported in rabbits exposed to 784 $\mu g/m^3$ (0.4 ppm) O_3, 6 hr/day, 5 days/week for 10 months (P'an et al., 1972). Thickened intima and media in pulmonary arterioles were reported in monkeys exposed to 1254 $\mu g/m^3$ (0.64 ppm) O_3, 8 hr/day for 1 year (Fujinaka et al., 1985).

Several of the effects of O_3 inhalation persisted after the O_3 inhalation ended and the animals breathed only filtered air several days or weeks. Lungs from rats exposed to 1568 $\mu g/m^3$ (0.8 ppm) O_3 for 72 hours appeared normal 6 days after the end of the exposure (Plopper et al., 1978). However, incomplete resolution of the nonciliated bronchiolar epithelial hyperplasia was reported in monkeys 7 days after 50 hours exposure to 1568 $\mu g/m^3$ (0.8 ppm) O_3 (Castleman et al., 1980) and in mice 10 days after a 20-day exposure to 1568 $\mu g/m^3$ (0.8 ppm) O_3, 24 hr/day (Ibrahim et al., 1980). Centriacinar inflammation and distal airway remodeling were still apparent 62 days after a 180-day exposure to 980 $\mu g/m^3$ (0.5 ppm) O_3, 24 hr/day (Moore and Schwartz, 1981).

While not all species of laboratory mammals have been studied following a single O_3 exposure regimen or using the same morphological techniques because investigators have asked different biological questions, there is a striking similarity of morphological effects in the respiratory system of all species studied. The cell types most damaged are the same. One of these cells, the type 1 alveolar epithelial cell, has a wide distribution in the pulmonary acinus and yet is damaged only in one specific location in all species studied. The other, the ciliated cell, appears damaged wherever it is located in the conducting airways. Damage to these cells is seen within hours after exposure to concentrations of O_3 much lower than 1 ppm and continues during exposures of weeks or months. Hyperplasia of other cell types is reported to start early in the exposure period, to continue throughout a long-term exposure, and when studied, to persist following postexposure periods of days or weeks. Centriacinar inflammation is also seen early and is reported throughout long exposure periods. Duration of centriacinar inflammation during postexposure periods has been studied less often and appears dependent upon length of the exposure period.

Other effects which have been reported in fewer studies or in a more limited number of species include distal airway remodeling and thickened pulmonary arteriolar walls. Remodeling of distal airways has only been reported in rats and monkeys after long-term exposures. In rats, remodeling of distal airways has been reported to persist for several weeks after the O_3 exposure has ended. Thickened pulmonary arteriolar walls have been reported only twice, once after long-term exposure of rabbits and once after long-term exposure of monkeys.

Studies on the morphologic effects of O_3 exposures of experimental animals are summarized in Figure 9-4 and Table 9-28 (see Section 9.6.1 for criteria used to summarize the studies).

9.6.3.2 Pulmonary Function. One of the limitations of animal studies is that many pulmonary function tests comparable to those conducted after acute exposure of human subjects are difficult to interpret. Methods exist, however, for obtaining similar measurements of many variables pertinent to understanding the effects of ozone on the respiratory tract, particularly after longer exposure periods. A number of newer studies reported here reflect recent advances in studying the effects of O_3 on pulmonary function in small animals.

Changes in lung function following ozone exposure have been studied in mice, rats, guinea pigs, rabbits, cats, dogs, sheep, and monkeys. Short-term exposure for 2 hr to concentrations of 431 to 980 $\mu g/m^3$ (0.22 to 0.5 ppm) produces rapid, shallow breathing and increased pulmonary resistance during exposure (Murphy et al., 1964; Yokoyama, 1969; Watanabe et al., 1973; Amdur et al., 1978). The onset of these effects is rapid and the abnormal breathing pattern usually disappears within 30 min after cessation of exposure. Other changes in lung function measured following short-term ozone exposures lasting 3 hr to 14 days are usually greatest 1 day following exposure and disappear by 7 to 14 days following exposure. These effects are associated with premature closure of the small, peripheral airways and include increased residual volume, closing volume, and closing capacity (Inoue et al., 1979).

Studies of airway reactivity following short-term ozone exposure of 1 to 2 hr duration in experimental animals show that O_3 increases the reactivity of

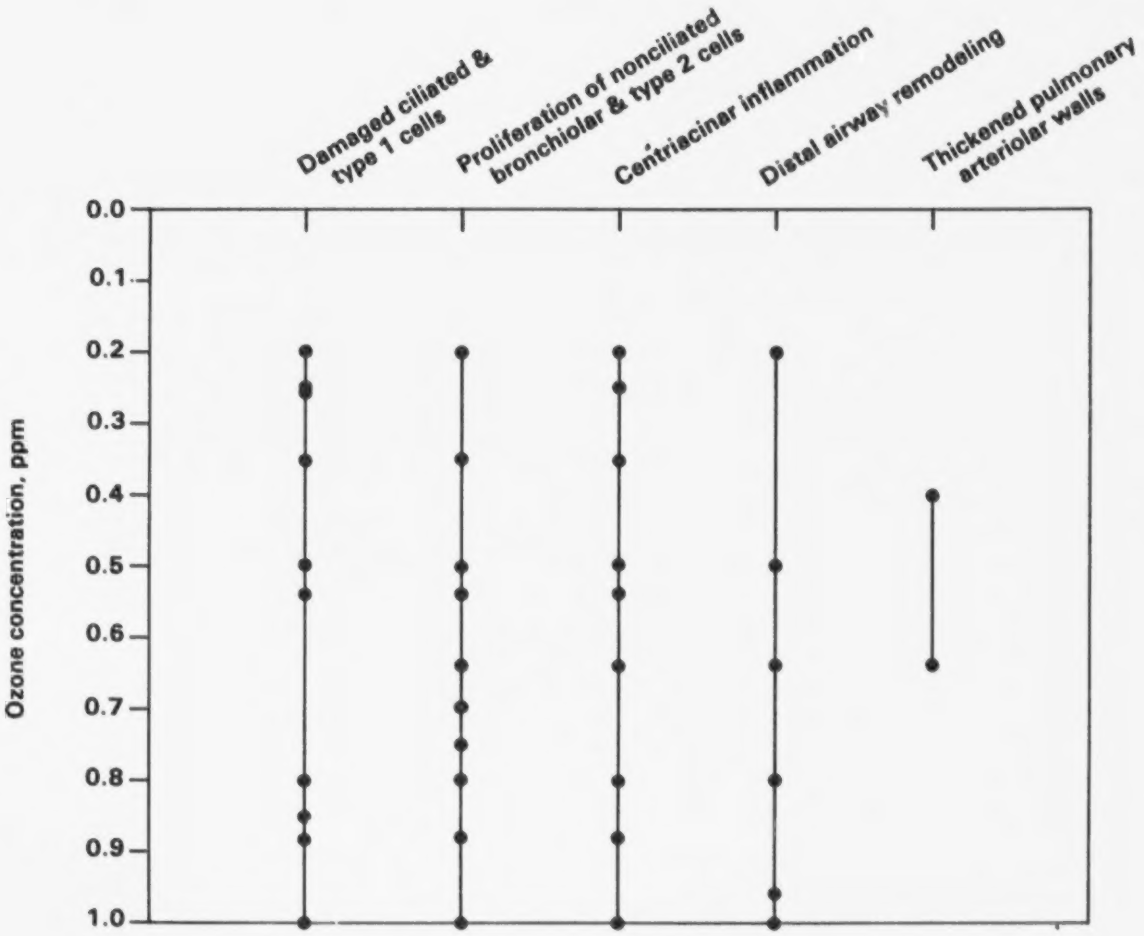

Figure 9-4. Summary of morphological effects in experimental animals exposed to ozone. See Table 9-28 for reference citations of studies summarized here.

TABLE 9-28. SUMMARY TABLE: MORPHOLOGICAL EFFECTS OF OZONE IN EXPERIMENTAL ANIMALS

Effect/response	O_3 concentration, ppm	References
Damaged ciliated and type 1 cells	[0.2], 0.5, 0.8	Boorman et al. (1980)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.2, 0.35	Castleman et al. (1977)
	0.25	Barry et al. (1983)
	0.25	Crapo et al. (1984)
	0.26, 0.50, 1.0	Boatman et al. (1974)
	0.5	Stephens et al. (1974b)
	0.5	Moore and Schwartz (1981)
	0.5	Evans et al. (1985)
	0.5, 0.8	Eustis et al. (1981)
	0.5, 0.8	Mellick et al. (1975, 1977)
	0.54, 0.88	Stephens et al. (1974a)
	0.8	Castleman et al. (1980)
	0.8	Plopper et al. (1978)
	0.85	Stephens et al. (1978)
Proliferation of nonciliated bronchiolar and type 2 cells	0.2, 0.35	Castleman et al. (1977)
	0.35, 0.50, 0.70, 0.75, 1.0	Evans et al. (1976b)
	0.5	Evans et al. (1985)
	0.5	Zitnik et al. (1978)
	0.5	Moore and Schwartz (1981)
	0.5, 0.8	Eustis et al. (1981)
	0.54, 0.88	Freeman et al. (1974)
	0.64	Fujinaka et al. (1985)
	0.7	Evans et al. (1976a)
	0.8	Castleman et al. (1980)
	0.8	Lum et al. (1978)
	0.8	Ibrahim et al. (1980)
	1.0	Cavender et al. (1977)
Centriacinar inflammation	[0.2], 0.5, 0.8	Boorman et al. (1980)
	0.2	Plopper et al. (1979)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.25	Barry et al. (1983)
	0.25	Crapo et al. (1984)
	0.35	Castleman et al. (1977)
	0.5	Stephens et al. (1974b)
	0.5	Moore and Schwartz (1981)
	0.5, 0.8	Mellick et al. (1975, 1977)
	0.5, 0.8	Brummer et al. (1977)
	0.5, 0.8	Last et al. (1979)
	0.54, 0.88	Stephens et al. (1974a)
	0.54, 0.88	Freeman et al. (1974)
	0.64	Fujinaka et al. (1985)
	0.8	Castleman et al. (1980)
	1.0	Freeman et al. (1973)

TABLE 9-28. SUMMARY TABLE: MORPHOLOGICAL EFFECTS OF OZONE IN EXPERIMENTAL ANIMALS (continued)

Effect/response	O_3 concentration, ppm	References
Distal airway remodeling	[0.2], 0.5, 0.8	Boorman et al. (1980)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.5	Moore and Schwartz (1981)
	0.64, 0.96	Last et al. (1984a)
	0.64	Fujinaka et al. (1985)
	1.0	Freeman et al. (1973)
Thickened pulmonary arteriolar walls	0.4	P'an et al. (1972)
	0.64	Fujinaka et al. (1985)

the lungs to a number of stimuli. Mild exercise, histamine aerosol inhalation, and breathing air with reduced oxygen or elevated carbon dioxide concentrations caused rapid, shallow breathing in conscious dogs immediately following 2-hr exposures to 1100 to 1666 $\mu g/m^3$ (0.56 to 0.85 ppm) of O_3 (Lee et al., 1979, 1980). Aerosolized ovalbumin caused an increased incidence of anaphylaxis in mice preexposed to 980 or 1568 $\mu g/m^3$ (0.5 or 0.8 ppm) of O_3 continuously for 3 to 5 days (Osebold et al., 1980). In addition, increased airway sensitivity to histamine or cholinomimetic drugs administered by aerosol or injection has been noted in several species after exposure to 980 to 5880 $\mu g/m^3$ (0.5 to 3.0 ppm) of O_3 (Easton and Murphy, 1967; Lee et al., 1977; Abraham et al., 1980, 1984a,b; Gordon and Amdur, 1980; Gordon et al., 1981, 1984; Roum and Murlas, 1984). The mechanism responsible for O_3-induced bronchial reactivity is still uncertain but may involve more than one specific factor. Ozone has been shown to cause increased sensitivity of vagal sensory endings in the dog airway (Lee et al., 1977, 1979, 1980). Ozone exposure may also enhance the airway responsiveness to bronchoconstrictors by altering sensitivity of the airway smooth muscle directly or through released cellular mediators (Gordon et al., 1981, 1984; Abraham et al., 1984a,b). In some species, increased airway hyperreactivity may be explained by increased transepithelial permeability or decreased thickness of the airway mucosa (Osebold et al., 1980; Abraham et al., 1984b). Ozone exposure may also decrease airway hyperreactivity by causing mucous hypersectetion, thereby limiting the airway penetration of inhaled bronchoconstrictors (Abraham et al., 1984a).

The time course of airway hyperreactivity after exposure to 980 to 5880 µg/m (0.5 to 3.0 ppm) of O_3 suggests a possible association with inflammatory cells and pulmonary inflammation (Holtzman et al., 1983a,b; Sielczak et al., 1983; Fabbri et al., 1984; O'Byrne et al., 1984a,b; Murlas and Roum, 1985). However, the time course of responsiveness is variable in different species and the relationships between airway inflammation and reactivity at different concentrations of O_3 are not well understood. Additional studies that demonstrate increased collateral resistance following 30 min local exposure of O_3 or histamine in sublobar bronchi of dogs (Gertner et al., 1983a,b,c,1984) suggest that other mechanisms, along with amplification of reflex pathways, may contribute to changes in airway reactivity depending not only on the concentration of O_3 in the airways but also on the extent of penetration of ozone into the lung periphery.

The effects of short-term exposures to O_3 on pulmonary function and airway reactivity in experimental animals are summarized in Figure 9-5 and Table 9-29 (see Section 9.6.1 for criteria used in developing this summary).

Exposures of 4 to 6 weeks to ozone concentrations of 392 to 490 µg/m^3 (0.2 to 0.25 ppm) increased lung distensibility at high lung volumes in young rats (Bartlett et al., 1974; Raub et al., 1983a). Similar increases in lung distensibility were found in older rats exposed to 784 to 1568 µg/m^3 (0.4 to 0.8 ppm) for up to 180 days (Moore and Schwartz, 1981; Costa et al., 1983; Martin et al., 1983). Exposure to O_3 concentrations of 980 to 1568 µg/m^3 (0.5 to 0.8 ppm) increased pulmonary resistance and caused impaired stability of the small peripheral airways in both rats and monkeys (Wegner, 1982; Costa et al., 1983; Yokoyama et al., 1984; Kotlikoff et al., 1984). The effects in monkeys were not completely reversed by 3 months following exposure; lung distensibility had also decreased in the postexposure period, suggesting the development of lung fibrosis which has also been suggested morphologically and biochemically.

The effects of long-term exposures to ozone on pulmonary function and airway reactivity in experimental animals are summarized in Figure 9-6 and Table 9-30 (see Section 9.6.1 for criteria used in developing this summary).

9.6.3.3 Biochemical Effects The lung is metabolically active, and several key steps in metabolism have been studied after O_3 exposure. Since the procedures for such studies are invasive, this research has been conducted only in animals. Effects, to be summarized below, have been observed on antioxidant metabolism, oxygen consumption, proteins, lipids, and xenobiotic metabolism.

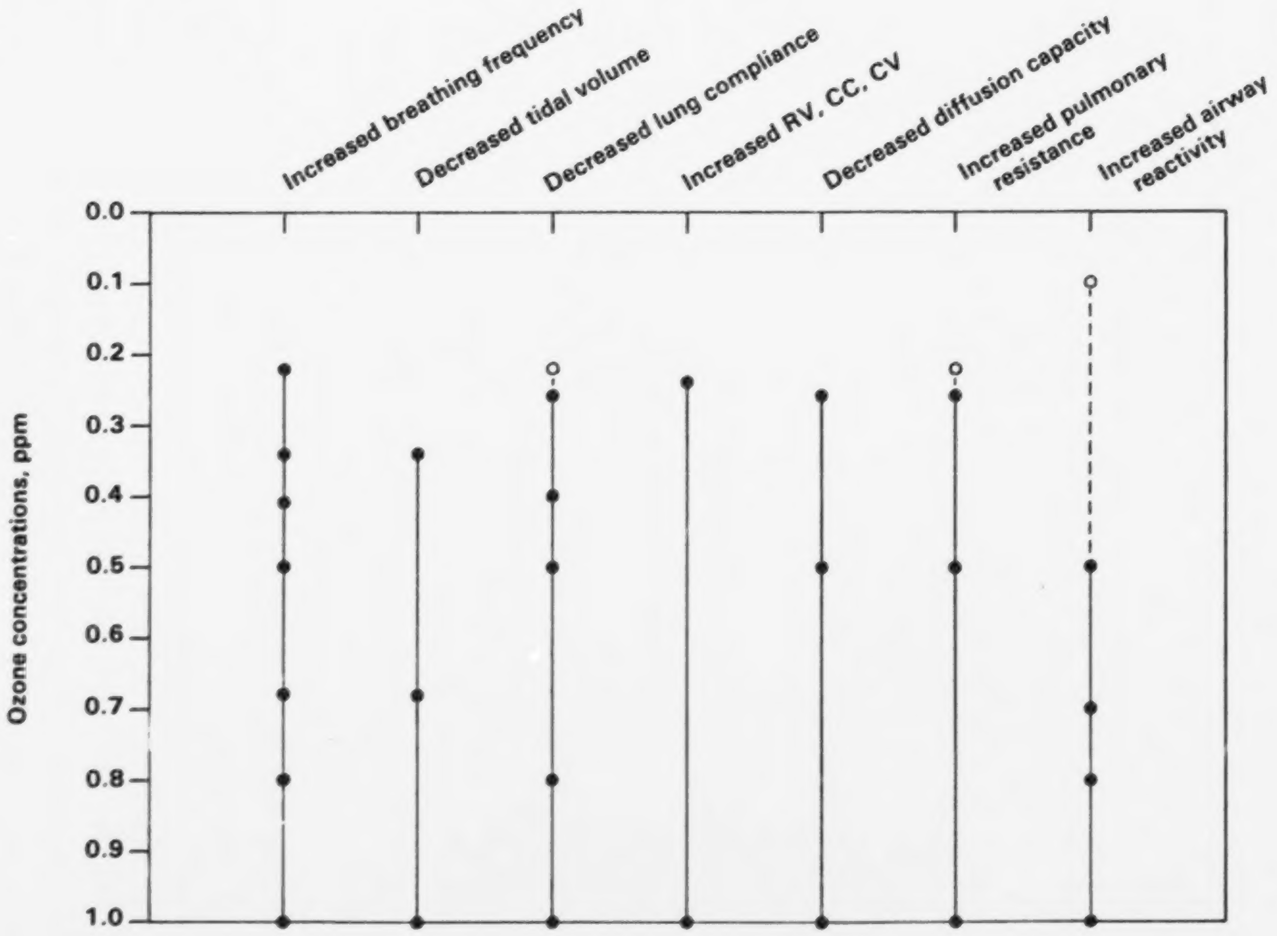

Figure 9-5. Summary of effects of short-term ozone exposures on pulmonary function in experimental animals. See Table 9-29 for reference citations of studies summarized here.

TABLE 9-29. SUMMARY TABLE: EFFECTS ON PULMONARY FUNCTION OF SHORT-TERM EXPOSURES TO OZONE IN EXPERIMENTAL ANIMALS

Effect/response	O_3 concentration, ppm	References
Increased breathing frequency	0.22, 0.41, 0.8 0.34, 0.68, 1.0 0.5	Amdur et al. (1978) Murphy et al. (1964) Yokoyama (1969)
Decreased tidal volume	0.34, 0.68, 1.0	Murphy et al. (1964)
Decreased lung compliance	[0.22], 0.41, 0.8 0.26, 0.5, 1.0 1.0	Amdur et al. (1978) Watanabe et al. (1973) Yokoyama (1974)
Increased residual volume (RV), closing capacity (CC), and closing volume (CV)	0.24 - 1.0	Inoue et al. (1979)
Decreased diffusion capacity	0.26, 0.5, 1.0	Watanabe et al. (1973)
Increased pulmonary resistance	[0.22] 0.26, 0.5, 1.0 0.5 1.0	Amdur et al. (1978) Watanabe et al. (1973) Yokoyama (1969) Yokoyama (1974)
Increased airway reactivity	[0.1]-0.8 [0.1]-0.8, 1.0 0.5, 1.0 0.7 1.0	Gordon and Amdur (1980) Gordon et al. (1981, 1984) Abraham et al. (1980, 1984a,b) Lee et al. (1977) Holtzman et al. (1983a,b)

The lung contains several compounds (e.g., vitamin E, sulfhydryls, glutathione) and enzymes (e.g., glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and superoxide dismutase) that function as antioxidants, thereby defending the lung against oxidant toxicity from the oxygen in air, from oxidants produced during metabolic processes, and from oxidizing air pollutants such as ozone. Obviously, this protection is only partial for O_3 since exposure to ozone causes numerous effects on lung structure, function, and biochemistry. Acute exposure to high ozone levels (2920 $\mu g/m^3$, 2 ppm) typically decreases antioxidant metabolism, whereas repeated exposures to lower levels (between 272 and 1568 $\mu g/m^3$, 0.2 and 0.8 ppm) increases this metabolism (DeLucia et al., 1975b). In rats maintained on normal

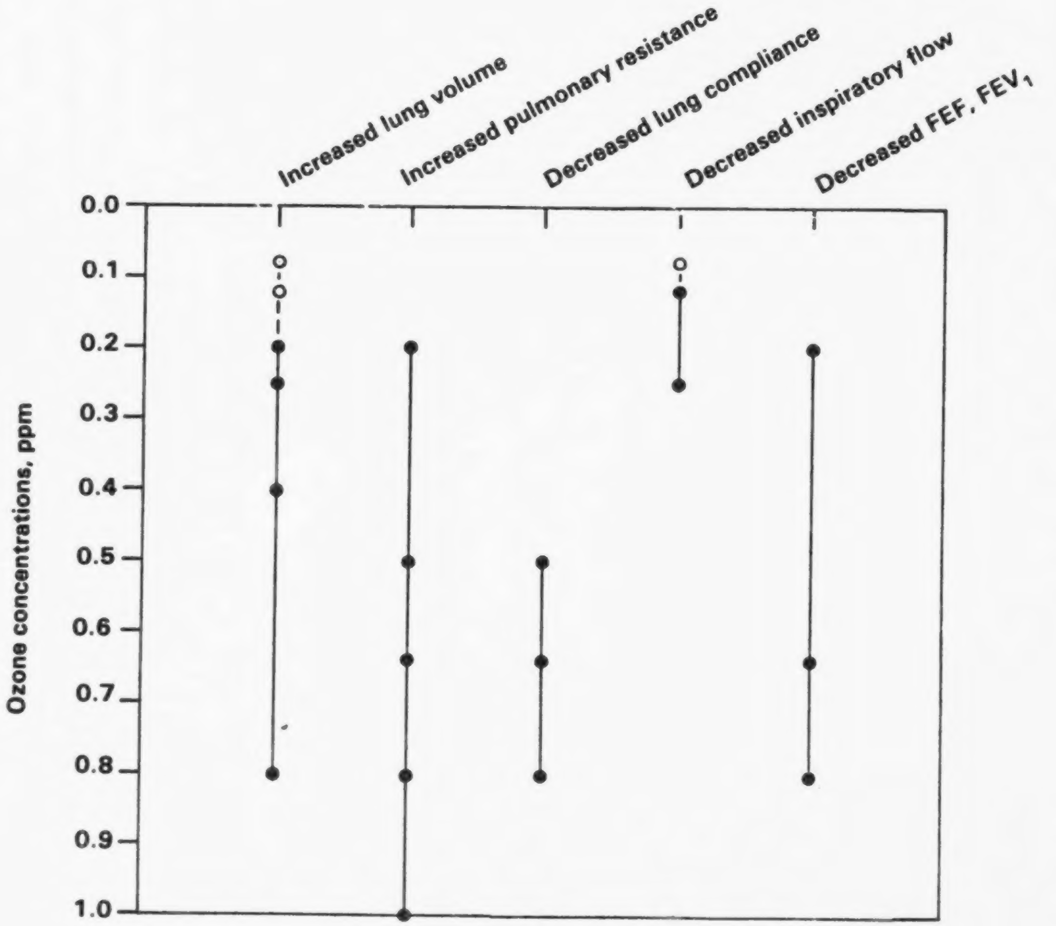

Figure 9-6. Summary of effects of long-term ozone exposures on pulmonary function in experimental animals. See Table 9-30 for reference citations of studies summarized here.

TABLE 9-30. SUMMARY TABLE: EFFECTS ON PULMONARY FUNCTION OF LONG-TERM EXPOSURES TO OZONE IN EXPERIMENTAL ANIMALS

Effect/response	O_3 concentration, ppm	References
Increased lung volume	[0.08], [0.12], 0.25	Raub et al. (1983a)
	0.2	Bartlett et al. (1974)
	[0.2], 0.8	Costa et al. (1983)
	0.4	Martin et al. (1983)
Increased pulmonary resistance	0.2, 0.8	Costa et al. (1983)
	0.5, 1.0	Yokoyama et al., 1984
	0.64	Wegner (1982)
	0.64	Kotlikoff et al., 1984
Decreased lung compliance	0.5, 0.8	Eustis et al. (1981)
	0.64	Wegner (1982)
Decreased inspiratory flow	[0.08], 0.12, 0.25	Raub et al. (1983)
Decreased forced expiratory volume (FEV_1) and flow (FEF)	0.2, 0.8	Costa et al. (1983)
	0.64	Wegner (1982)

diets, this response has been observed after a week of continuous or intermittent exposure to 392 $\mu g/m^3$ (0.2 ppm) O_3 (Mustafa, 1975; Mustafa and Lee, 1976; Plopper et al., 1979). Similar responses are seen in monkeys and mice, but at higher concentrations (980 $\mu g/m^3$, 0.5 ppm) (Fukase et al., 1978; Mustafa and Lee, 1976).

The effects of O_3 on oxygen consumption have been studied since oxygen consumption is a fundamental parameter of cellular metabolism, reflecting energy production by cells. As with antioxidant metabolism, acute exposure to high ozone levels ($\geq$ 3920 $\mu g/m^3$; $\geq$ 2 ppm) decreases metabolism (and thus, oxygen consumption); repeated exposure to lower levels (> 1568 $\mu g/m^3$, 0.8 ppm) increases oxygen consumption (Mustafa et al., 1973; Schwartz et al., 1976; Mustafa and Lee, 1976). Effects in rats on normal diets have been observed after a short-term exposure to ozone levels as low as 392 $\mu g/m^3$ (0.2 ppm) (Schwartz et al., 1976; Mustafa et al., 1973; Mustafa and Lee, 1976). Monkeys are affected at a higher level of ozone (980 $\mu g/m^3$, 0.5 ppm).

Similar patterns of response for both antioxidant metabolism and oxygen consumption are observed after exposure to ozone. A 7-day exposure to ozone produces linear concentration-related increases in activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and succinate oxidase (Mustafa and Lee, 1976; Chow et al., 1974; Schwartz et al., 1976; Mustafa et al., 1973). Rats on a vitamin E-deficient diet experience an increase in enzyme activities at 196 $\mu g/m^3$ (0.1 ppm) ozone as compared to 392 $\mu g/m^3$ (0.2 ppm) in animals on normal diets (Chow et al., 1981; Mustafa and Lee, 1976; Mustafa, 1975). Research on these enzymes has shown that there is no significant difference in effects from continuous versus intermittent exposure; this, along with concentration-response data, suggests that the concentration of ozone is more important than duration of exposure in causing these effects (Chow et al., 1974; Schwartz et al., 1976; Mustafa and Lee, 1976).

Duration of exposure still plays a role, however. During exposures up to 1 or 4 weeks, antioxidant metabolism and O_2 consumption generally do not change on the first day of exposure; by about day 2, increases are observed and by about day 4 a plateau is reached (Mustafa and Lee, 1976; DeLucia et al., 1975a). Recovery from these effects occurs by 6 days post-exposure (Chow et al., 1976b). This plateauing of effects in the presence of exposure does not result in long-term tolerance. If rats are re-exposed after recovery is observed, the increase in enzyme activities is equivalent to that observed in animals exposed for the first time (Chow et al., 1976b).

The influence of age on responsiveness is also similar for antioxidant metabolism and oxygen consumption (Elsayed et al., 1982a; Tyson et al., 1982; Lunan et al., 1977). Suckling neonates (5 to 20 days old) generally exhibited a decrease in enzyme activities; as the animals grew older (up to about 180 days old), enzyme activities generally increased with age. Species differences may exist in this response (Mustafa and Lee, 1976; Mustafa et al., 1982; Chow et al., 1975; DeLucia et al., 1975a). Studies in which monkeys have been compared to rats did not include a description of appropriate statistical considerations applied (if any); thus, no definitive conclusions about responsiveness of monkeys versus rats can be made.

The mechanism responsible for the increase in antioxidant metabolism and oxygen consumption is not known. The response is typically attributed, however, to concurrent morphological changes, principally the loss of type 1 cells and an increase in type 2 cells that are richer in the enzymes measured.

Monooxygenases constitute another class of enzymes investigated after ozone exposure. These enzymes function in the metabolism of both endogenous (e.g., biogenic amines, hormones) and exogenous (xenobiotic) substances. The substrates acted upon are either activated or detoxified, depending on the substrate and the enzyme. Acute exposure to 1470 to 1960 μg/m^3 (0.75 to 1 ppm) ozone decreased cytochrome P-450 levels and enzyme activities related to both cytochrome P-450 and P-448. The health impact of these changes is uncertain since only a few elements of a complex metabolic system were measured.

The activity of lactate dehydrogenase is increased in lungs of vitamin E-deficient rats receiving a short-term exposure to 196 μg/m^3 (0.1 ppm) ozone (Chow et al., 1981). Higher levels caused a similar response in rats, but not in monkeys, on normal diets (Chow et al., 1974, 1977). This enzyme is frequently used as a marker of cellular damage because it is released upon cytotoxicity. It is not known, however, whether the increase in this enzyme is a direct reflection of cytotoxicity or whether it is an indicator of an increased number of type 2 cells and macrophages in the lungs.

An increase in a few of the measured activities of lysosomal enzymes has been shown in the lungs of rats exposed to $\geq$ 1372 μg/m^3 (0.7 ppm) ozone (Dillard et al., 1972; Castleman et al., 1973a; Chow et al., 1974). This response is most likely the result of an increase in inflammatory cells in the lungs rather than an induction of enzymes, since lysosomal enzymes in alveolar macrophages decrease after *in vivo* or *in vitro* exposure to ozone (Hurst et al., 1970; Hurst and Coffin, 1971).

As discussed previously, long-term exposure to high O_3 concentrations causes mild lung fibrosis (i.e., local increase of collagen in centriacinar interalveolar septa). This morphological change has been correlated with biochemical changes in the activity of prolyl hydroxylase (an enzyme that catalyzes the production of hydroxyproline) and in hydroxyproline content (a component of collagen that is present in excess in fibrosis) (Last et al., 1979; Bhatnagar et al., 1983). An increase in collagen synthesis has been observed, with 980 μg/m^3 (0.5 ppm) O_3 being the minimally effective concentration tested (Hussain et al., 1976a,b; Last et al., 1979). During a prolonged exposure, prolyl hydroxylase activity increases by day 7 and returns to control levels by 60 days of exposure. When a short-term exposure ceases, prolyl hydroxylase activity returns to normal by about 10 days post-exposure, but hydroxyproline levels remain elevated 28 days post-exposure. Thus, the product of the increased synthesis, collagen, remains relatively stable. One study (Costa et al., 1983) observed a small decrease in collagen levels of rats at 392 and 1568 μg/m^3 (0.2 and 0.8 ppm) O_3 after an intermittent exposure for 62 days.

The effects of O_3 on increasing collagen content may be progressive; i.e., after a 6-week intermittent exposure of rats to 0.64 or 0.96 ppm O_3 ceased, collagen levels 6 week post-exposure were elevated over the levels immediately after exposure (Last et al., 1984b). Also, there appears to be little difference between continuous and intermittent exposure in increasing collagen levels in rat lungs (Last et al, 1984b). Thus, the intermittent clean air periods were not sufficient to permit recovery.

Although the ability of O_3 to initiate peroxidation of unsaturated fatty acids _in vitro_ is well established, few _in vivo_ studies of lung lipids have been conducted. Generally, ozone decreases unsaturated fatty acid content of the lungs (Roehm et al., 1972) and decreases incorporation of fatty acids into lecithin (a saturated fatty acid) (Kyei-Aboagye et al., 1973). These alterations, however, apparently do not alter the surface-tension-lowering properties of lung lipids that are important to breathing (Gardner et al., 1971; Huber et al., 1971).

One of the earliest demonstrated effects of ozone was that very high concentrations caused mortality as a result of pulmonary edema. As more sensitive techniques were developed, lower levels (510 μg/m^3, 0.26 ppm) were observed to increase the protein content of the lung (Hu et al., 1982). Since some of the excess protein could be attributed to serum proteins, the interpretation was that edema had occurred. This effect was more pronounced several hours after exposure ceased. At higher concentrations, a loss of carrier-mediated transport from the air side of the lung to the blood side was observed (Williams et al., 1980). These changes imply an effect on the barrier function of the lung, which regulates fluxes of various substances with potential physiological activities across the alveolar walls.

The biochemical effects observed in experimental animals exposed to O_3 are summarized in Figure 9-7 and Table 9-31 (see Section 9.6.1 for criteria used in developing this summary).

9.6.3.4 Host Defense Mechanisms. Reports over the years have presented substantial evidence that exposure to ozone impairs the antibacterial activity of the lung, resulting in an impairment of the lung's ability to kill inhaled

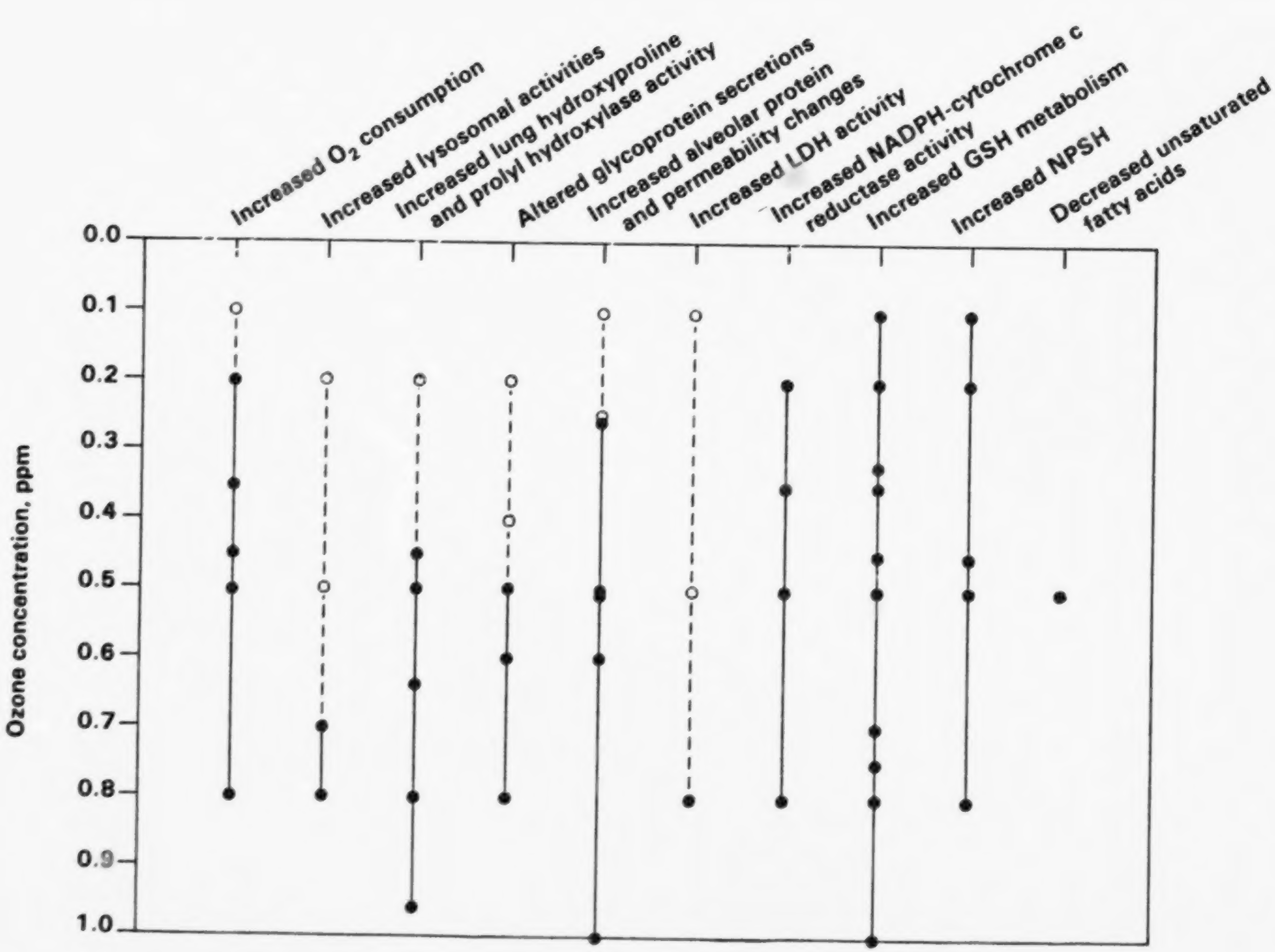

Figure 9-7. Summary of biochemical changes in experimental animals exposed to ozone. See Table 9-31 for reference citations of studies summarized here.

TABLE 9-31. SUMMARY TABLE: BIOCHEMICAL CHANGES IN EXPERIMENTAL ANIMALS EXPOSED TO OZONE

Effect/response	O_3 concentration, ppm	References
Increased O_2 consumption	[0.1], 0.2	Mustafa (1975)
	[0.1], 0.2, 0.35, 0.5, 0.8	Mustafa and Lee (1976)
	0.2, 0.5, 0.8	Mustafa et al. (1973)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.45	Mustafa et al. (1982)
	0.8	Chow et al. (1976b)
	0.8	Elsayed et al. (1982a)
Increased lysosomal enzyme activities	[0.2], [0.5], 0.8	Chow et al. (1974)
	0.7, 0.8	Dillard et al. (1972)
	0.7, 0.8	Castleman et al. (1973a,b)
Increased lung hydroxyproline and prolyl hydroxylase activity	[0.2], 0.5, 0.8	Hussain et al. (1976a,b)
	0.2, 0.8	Costa et al. (1983)
	0.45, 0.8	Bhatnagar et al. (1983)
	0.5, 0.64, 0.96	Last et al. (1979, 1984b)
	0.5	Last and Greenberg (1980)
	0.8	Hesterberg and Last (1981)
Altered mucus glycoprotein secretions	[0.2], [0.4], 0.5, 0.6, 0.8	Last and Kaizu (1980)
	0.5, 0.6, 0.8	Last and Cross (1978)
	0.6, 0.8	Last et al. (1977)
Increased alveolar protein and permeability changes	[0.1], 0.26, 0.51, 1.0	Hu et al. (1982)
	[0.25], 0.5, 1.0	Alpert et al. (1971a)
	0.6, 1.0	Williams et al. (1980)
	1.0	Reasor et al. (1979)
Increased LDH activity	[0.1]	Chow et al. (1981)
	[0.5], 0.8	Chow et al. (1977)
	0.8	Chow and Tappel (1973)
Increased NADPH - cytochrome c reductase activity	0.2, 0.35, 0.8	Mustafa and Lee (1976)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.2, 0.5, 0.8	DeLucia et al. (1972, 1975a,b)
Increased GSH metabolism	[0.1]	Chow et al. (1981)
	0.1, 0.2	Plopper et al. (1979)
	0.2, 0.35, 0.5, 0.8	Mustafa and Lee (1976)
	0.2, 0.5, 0.8	Chow et al. (1974)
	0.2, 0.5, 0.8	DeLucia et al. (1972, 1975a,b)
	0.2, 0.5, 0.8	Schwartz et al. (1976)
	0.2, 0.5, 1.0	Fukase et al. (1975)
	0.32	Moore et al. (1980)
	0.45	Mustafa et al. (1982)
	0.5	Chow et al. (1975)

TABLE 9-31. SUMMARY TABLE: BIOCHEMICAL CHANGES IN EXPERIMENTAL ANIMALS EXPOSED TO OZONE (continued)

Effect/response	O_3 concentration, ppm	References
	0.5, 1.0	Fukase et al. (1978)
	0.7, 0.75, 0.8	Chow and Tappel (1972, 1973)
	0.8	Elsayed et al. (1982a,b; 1983)
	0.8	Chow et al. (1976b)
	0.9	Tyson et al. (1982)
	0.9	Lunan et al. (1977)
Increased NPSH	0.1, 0.2	Plopper et al. (1979)
	0.2, 0.5, 0.8	DeLucia et al. (1975b)
	0.45	Mustafa et al. (1982)
	0.8	Chow et al. (1976b)
Decreased unsaturated fatty acids	0.5	Roehm et al., 1972

microorganisms. Suppression of this biocidal defense of the lung can lead to microbial proliferation within the lung, resulting in mortality. The mortality response is concentration-related and is significant at concentrations as low as 157 to 196 $\mu g/m^3$ (0.08 to 0.1 ppm) (Coffin et al., 1967; Ehrlich et al., 1977; Miller et al., 1978a; Aranyi et al., 1983). The biological basis for this response appears to be that ozone or one of its reactive products can impair or suppress the normal bactericidal functions of the pulmonary defenses, which results in prolonging the life of the infectious agent, permitting its multiplication and ultimately, in this animal model, resulting in death. Such infections can occur because of O_3 effects on a complex host defense system involving alveolar macrophage functioning, lung fluids, and other immune factors.

The data obtained in various experimental animal studies indicate that short-term ozone exposure can reduce the effectiveness of several vital defense systems including (1) the ability of the lung to inactivate bacteria and viruses (Coffin et al., 1968; Coffin and Gardner, 1972b; Goldstein et al., 1974a, 1977; Warshauer et al., 1974; Bergers et al; 1983. Schwartz and Christman, 1979; Ehrlich et al., 1979); (2) the mucociliary transport system (Phalen et al., 1980; Frager et al., 1979; Kenoyer et al., 1981; (3) the immunological system (Campbell and Hilsenroth, 1976; Fujimaki et al., 1984; Thomas et al., 1981b; Aranyi et al., 1983; and (4) the pulmonary macrophage (Dowell et al., 1970; Goldstein et al., 1971a,b, and 1977; Hadley et al., 1977; McAllen et al., 1981; Witz et al., 1983; Hurst et al., 1970; Hurst and Coffin, 1971; Amoruso et al., 1981). Studies have also indicated that the activity level of the test subject and the presence of other airborne chemicals are important variables that can influence the determination of the lowest effective concentration of the pollutant (Gardner et al., 1977; Aranyi et al., 1983; Ehrlich, 1980, 1983; Grose et al., 1980, 1982; Phalen et al., 1980; Goldstein et al., 1974a; Illing et al., 1980).

Ciliated cells are damaged by O_3 inhalation, as demonstrated by major morphological changes in these cells, including necrosis and sloughing, or by the shortening of the cilia in cells attached to the bronchi. Sufficient ciliated cell damage should result in decreased transport of viable and nonviable particles from the lung. Rats exposed to 784, 1568, 1960, or 2352 $\mu g/m^3$ (0.4, 0.8, 1.0, or 1.2 ppm) for times as short as 4 hr have decreased short-term clearance of particles from the lung (Phalen et al., 1980; Frager et al., 1979; Kenoyer et al., 1981). Short-term clearance is mostly due to mucus transport of particles, and the decreased short-term clearance is an anticipated functional result predicted from morphological observations. The mucous glycoprotein production of the trachea is also altered by O_3 exposure. Mucous glycoprotein biosynthesis, as measured *ex vivo* in cultured tracheal explants from exposed rats, was inhibited by short-term continuous exposure to 1568 $\mu g/m^3$ (0.8 ppm) of O_3 for 3 to 5 days (Last and Cross, 1978; Last and Kaizu, 1980; Last et al., 1977). Glycoprotein synthesis and secretion recovered to control values after 5 to 10 days of exposure and increased to greater than control values after 10 days of exposure. With this increase in production of mucus, investigators have found that the velocity of the tracheal mucus was significantly reduced following a 2 hr exposure to 1960 $\mu g/m^3$ (1.0 ppm) (Abraham et al., 1980).

A problem remains in assessing the relevance of these animal data to humans. Green (1984) reviewed the literature and compared the host antibacterial defense systems of the rodent and man and found that these two species had defenses that are very similar and thus provide a good basis for a qualitative extrapolation. Both defenses consist of an aerodynamic filtration system, a fluid layer lining the respiratory membranes, a transport mechanism for removing foreign particles, microorganisms, and pulmonary cells, and immune secretions of lymphocytes and plasma cells. In both rodents and humans, these components act in concert to maintain the lung free of bacteria.

If the animal models are to be used to reflect the toxicological response occurring in humans, then the endpoint for comparison of such studies should be morbidity rather than mortality. A better index of O_3 effect in humans might be the increased prevalence of infectious respiratory illness in the community. Such a comparison may be proper since both mortality from respiratory infections (animals) and morbidity from respiratory infections (humans) can result from a loss in pulmonary defenses (Gardner, 1984). Whether the microorganisms used in the various animal studies are comparable to the organisms responsible for the respiratory infections in a community still requires further investigation.

Ideally, studies of pulmonary host defenses should be performed in man, using epidemiological or volunteer methods of study. Unfortunately, such studies have not been reported yet. Attention must therefore be paid to the results of host-defense experiments conducted with animals.

In the area of host defense of the lung against infection, present knowledge of the physiology, metabolism, and function have come primarily from the study of various animal systems, but it is generally accepted that the basic mechanisms of action of these defense cells and systems function similarly in both animals and man. There are also human data to support this statement, especially in such areas as immunosuppression, ciliostasis, and alveolar macrophages. The effects seen in animals represent alterations in basic biological systems. One can assume that similar alterations in basic defense mechanisms could occur in humans since they possess equivalent pulmonary defense systems. It is understood, however, that different exposure levels may be required to produce similar responses in humans. The concentration of O_3 at which effects become evident can be influenced by a number of factors, such as preexisting disease, virulence of the infectious agent, dietary factors, concurrent exposure to other pollutants, exercise, or the presence of other environmental stresses, or a combination of these. Thus, one could hypothesize that humans exposed to O_3 could experience effects on host defense mechanisms. At the present time, however, one cannot predict the exact concentration at which effects may occur in man nor the severity of the effects.

The effects of O_3 on host defense mechanisms in experimental animals are summarized in Figure 9-8 and Table 9-32 (see Section 9.6.1 for criteria used in developing this summary).

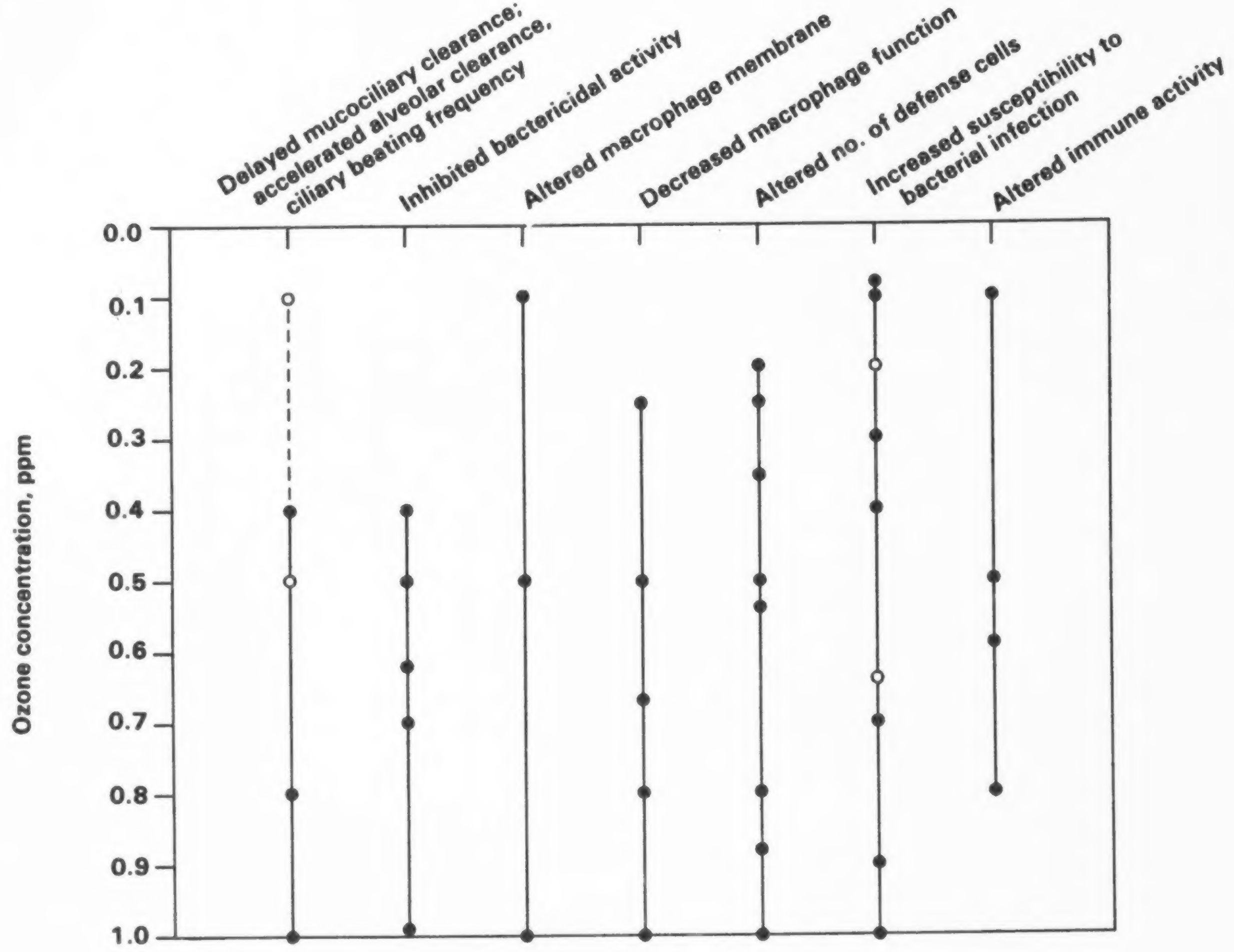

Figure 9-8. Summary of effects of ozone on host defense mechanisms in experimental animals. See Table 9-32 for reference citations of studies summarized here.

TABLE 9-32. SUMMARY TABLE: EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS IN EXPERIMENTAL ANIMALS

Effect/response	O_3 concentration, ppm	References
Delayed mucociliary clearance; accelerated alveolar clearance, ciliary beating frequency	[0.1]	Grose et al. (1980)
	0.4, 0.8, 1.0	Kenoyer et al. (1981)
	[0.5]	Friberg et al. (1972)
	[0.5], 1.0	Abraham et al. (1980)
	0.8	Phalen et al. (1980)
	1.2	Frager et al. (1979)
Inhibited bactericidal activity	0.4	Coffin and Gardner (1972b)
	0.4	Goldstein et al. (1972b)
	0.5	Friberg et al. (1972)
	0.62	Goldstein et al. (1971b)
	0.7	Bergers et al. (1983)
	0.7	Warshauer et al. (1974)
	0.99	Goldstein et al. (1971a)
Altered macrophage membrane	0.1, 1.0	Gardner et al. (1971)
	0.5	Dowell et al. (1970)
	0.5	Hadley et al. (1977)
	0.5, 1.0	Goldstein et al. (1977)
Decreased macrophage function	0.25, 0.5	Hurst et al. (1970) Hurst and Coffin (1971)
	0.5	Alpert et al. (1971b)
	0.5, 0.67	Coffin et al. (1968)
	0.5, 0.67	Coffin and Gardner (1972b)
	0.8	Schwartz and Christman (1979)
	1.0	Shingu et al. (1980)
	1.0	McAllen et al. (1981)
Altered no. of defense cells	0.2	Plopper et al. (1979)
	0.2, 0.35, 0.5, 0.8	Dungworth et al. (1975b)
	0.2, 0.35	Castleman et al. (1977)
	0.2, 0.5, 0.8	Boorman et al. (1977, 1980)
	0.25	Barry et al. (1983)
	0.5	Zitnik et al. (1978)
	0.5, 0.88	Stephens et al. (1974a)
	0.5	Last et al. (1979)
	0.5, 0.88	Brummer et al. (1977)
	0.5, 0.8	Eustis et al. (1981)
	0.54, 0.88	Freeman et al. (1974)
	0.8	Castleman et al. (1980)
	1.0	Freeman et al. (1973)
	1.0	Cavender et al. (1977)
Increased susceptibility to infection	0.08	Coffin et al. (1967)
	0.08, 0.1	Miller et al. (1978a)
	0.1	Ehrlich et al. (1977)

TABLE 9-32. SUMMARY TABLE: EFFECTS OF OZONE ON HOST DEFENSE MECHANISMS IN EXPERIMENTAL ANIMALS (continued)

Effect/response	O_3 concentration, ppm	References
Increased susceptibility (cont'd)	0.1	Aranyi et al. (1983)
	0.1, 0.3	Illing et al. (1980)
	[0.2], 0.4, 0.7	Bergers et al. (1983)
	0.3	Abraham et al. (1982)
	0.5	Wolcott et al. (1982)
	[0.64]	[Sherwood et al. (1984)]
	0.7, 0.9	Coffin and Blommer (1970)
	1.0	Thomas et al. (1981b)
Altered immune activity	0.1	Aranyi et al. (1983)
	0.5, 0.8	Osebold et al. (1979, 1980)
	0.5, 0.8	Gershwin et al. (1981)
	0.59	Campbell and Hilsenroth (1976)
	0.8	Fujimaki et al. (1984)

9.6.3.5 Tolerance. Examination of responses to short-term, repeated exposures to O_3 clearly indicates that with some of the parameters measured, animals have an increased capacity to resist the effects of subsequent exposure. This tolerance persists for varying times, depending on the degree of development of the tolerance. Previous exposure to low concentrations of O_3 will protect against the effects of subsequent exposure to lethal doses and the development of lung edema (Stokinger et al., 1956; Fairchild, 1967; Coffin and Gardner, 1972a; Chow, 1984). The prolongation of mucociliary clearance reported for O_3 can also be eliminated by pre-exposure to a lower concentration (Frager et al., 1979). This effect is demonstrated for a short period of time and is lost as soon as the mucus secretion rate returns to normal. However, not all of the toxic effects of O_3, such as reduced functioning activity of the pulmonary defense system (Gardner et al., 1972); hyperplasia of the type 2 cells (Evans et al., 1971, 1976a,b); increased susceptibility to respiratory disease (Gardner and Graham, 1977); loss of pulmonary enzymatic activity (Chow, 1976, Chow et al., 1976b); and inflammatory response (Gardner et al., 1972) can be totally prevented by prior treatment with low levels of O_3. Dungworth et al. (1975b) and Castleman et al. (1980) have attempted to explain tolerance by careful examination of the morphological changes that occur with repeated O_3 exposures. These investigators suggest that during continuous exposure to O_3 the injured cells attempt to initiate early repair of the specific lesion. The repair phase results in a reduction of the effect first observed but lasts only for a short time since the recovered cells are as sensitive to re-exposure to O_3 as the pre-exposed counterpart (Plopper et al., 1978). This information is an important observation because it implies that the decrease in susceptibility to O_3 persists only as long as the exposure to O_3 continues. The biochemical studies of Chow et al. (1976b) support this conclusion.

At this time, there are a number of hypotheses proposed to explain the mechanism of this phenomenon (Mustafa and Tierney, 1978; Schwartz et al., 1976; Mustafa et al., 1977; Berliner et al., 1978; Gertner et al., 1983b; Bhatnagar et al., 1983). Evidence by Nambu and Yokoyama (1983) indicates that although the pulmonary antioxidant system (glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase) may play an active role in defending the lung against ozone, it does not explain the mechanism of tolerance in that the development of tolerance does not coincide with the described biochemical enhancement of the antioxidant system in the lungs of rats.

From this literature, it would appear that tolerance, as seen in animals, may not be the result of any one single biological process, but instead may result from a number of different events, depending on the specific response measured. Tolerance does not imply complete or absolute protection, because continuing injury does still occur, which could potentially lead to nonreversible pulmonary changes.

Tolerance may not be long-lasting. During O_3 exposure, the increase in antioxidant metabolism reaches a plateau and recovery occurs a few days after exposure ceases. Upon re-exposure, effects observed are similar to those that occurred during the primary exposure (Chow et al., 1976b).

9.6.4 Extrapulmonary Effects of Ozone

It is still believed that O_3, on contact with respiratory system tissue, immediately reacts and thus is not absorbed or transported to extrapulmonary sites to any significant degree. However, several studies suggest that possibly products formed by the interaction of O_3 and respiratory system fluids or tissue can produce effects in lymphocytes, erythrocytes, and serum, as well as in the parathyroid gland, the heart, the liver, and the CNS. Ozone exposure also produces effects on animal behavior that may be caused by pulmonary consequences of O_3, or by nonpulmonary (CNS) mechanisms. The mechanism by which O_3 causes extrapulmonary changes is unknown. Mathematical models of O_3 dosimetry predict that very little O_3 penetrates to the blood of the alveolar capillaries. Whether these effects result from O_3 or a reaction product of O_3 which penetrates to the blood and is transported is the subject of speculation.

9.6.4.1 Central Nervous System and Behavioral Effects. Ozone significantly affects the behavior of rats during exposure to concentrations as low as 235 $\mu g/m^3$ (0.12 ppm) for 6 hr. With increasing concentrations of O_3, further decreases in unspecified motor activity and in operant learned behaviors have been observed (Konigsberg and Bachman, 1970; Tepper et al., 1982; Murphy et al., 1964; and Weiss et al., 1981). Tolerance to the observed decrease in motor activity may occur on repeated exposure. At low O_3 exposure concentrations (490 $\mu g/m^3$, 0.25 ppm), an increase in activity is observed after exposure ends. Higher O_3 concentrations (980 $\mu g/m^3$, 0.5 ppm) produce a decrease in rodent activity that persists for several hours after the end of exposure (Tepper et al., 1982, 1983).

The mechanism by which behavioral performance is reduced is unknown. Physically active responses appear to enhance the effects of O_3, although this may be the result of an enhanced minute volume that increases the effective concentration delivered to the lung. Several reports indicate that it is unlikely that animals have reduced physiological capacity to respond, prompting Weiss et al. (1981) to suggest that O_3 impairs the inclination to respond. Two studies indicate that mice will respond to remove themselves from an atmosphere containing greater than 980 $\mu g/m^3$ (0.5 ppm) (Peterson and Andrews, 1963, Tepper et al., 1983). These studies suggest that the aversive effects of O_3 may be due to lung irritation. It is unknown whether lung irritation, odor, or a direct effect on the CNS causes change in rodent behavior at lower O_3 concentrations.

9.6.4.2 Cardiovascular Effects. Studies on the effects of O_3 on the cardiovascular system are few, and to date there are no reports of attempts to confirm these studies. The exposure of rats to O_3 alone or in combination with cadmium (1176 $\mu g/m^3$, 0.6 ppm O_3) resulted in measurable increases in systolic pressure and heart rate (Revis et al., 1981). No additive or antagonistic response was observed with the combined exposure. Pulmonary capillary blood flow and PaO_2 decreased 30 min following exposure of dogs to 588 $\mu g/m^3$ (0.3 ppm) of O_3 (Friedman et al., 1983). The decrease in pulmonary capillary blood flow persisted for as long as 24 hr following exposure.

9.6.4.3 Hematological and Serum Chemistry Effects. The data base for the effects of O_3 on the hematological system is extensive and indicates that O_3 or one of its reactive products can cross the blood-gas barrier, causing changes in the circulating erythrocytes (RBC) as well as significant differences in various components of the serum.

Effects of O_3 on the circulating RBCs can be readily identified by examining either morphological and/or biochemical endpoints. These cells are structually and metabolically well understood and are available through relatively non-invasive methods, which makes them ideal candidates for both human and animal studies. A wide range of structural effects have been reported in a variety of species of animals, including an increase in the fragility of RBCs isolated from monkeys exposed to 1470 $\mu g/m^3$ (0.75 ppm) of O_3 4 hr/day for 4 days (Clark et al., 1978). A single 4-hr exposure to 392 $\mu g/m^3$ (0.2 ppm) also caused increased fragility as well as sphering of RBCs of rabbits (Brinkman et al., 1964). An increase in the number of RBCs with Heinz bodies was detected following a 4-hr exposure to 1666 $\mu g/m^3$ (0.85 ppm). The presence of such inclusion bodies in RBCs is an indication of oxidant stress (Menzel et al., 1975a).

These morphological changes are frequently accompanied by a wide range of biochemical effects. RBCs of monkeys exposed to 1470 $\mu g/m^3$ (0.75 ppm) of O_3 for 4 days also had a decreased level of glutathione (GSH) and decreased acetylcholinesterase (AChE) activity, an enzyme bound to the RBC membranes. The RBC GSH activity remained significantly lower 4 days postexposure (Clark et al., 1978).

Animals deficient in vitamin E are more sensitive to O_3. The RBCs from these animals, after being exposed to O_3, had a significant increase in the activity of GSH peroxidase, pyruvate kinase, and lactic dehydrogenase, but had a decrease in RBC GSH after exposure to 1568 $\mu g/m^3$ (0.8 ppm) for 7 days (Chow and Kaneko, 1979). Animals with a vitamin E-supplemented diet did not have any changes in glucose-6-phosphate dehydrogenase (G-6-PD), superoxide dismutase, or catalase activities. At a lower level (980 $\mu g/m^3$, 0.5 ppm), there were no changes in GSH level or in the activities of GSH peroxidase or GSH reductase (Chow et al., 1975). Menzel et al. (1972) also reported a significant increase in lysis of RBCs from vitamin E-deficient animals after 23 days of exposure to 980 $\mu g/m^3$ (0.5 ppm). These effects were not observed in vitamin E-supplemented rats. Mice on a vitamin E-supplemented diet and those on a deficient diet showed an increase in G-6-PD activity after an exposure of 627 μg/m^3 (0.32 ppm) of O_3 for 6 hr. Decreases observed in AChE activity occurred in both groups (Moore et al., 1980).

Other blood changes are attributed to O_3. Rabbits exposed for 1 hr to 392 μg/m^3 (0.2 ppm) of O_3 showed a significant drop in total blood serotonin (Veninga, 1967). Six- and 10-month exposures of rabbits to 784 μg/m^3 (0.4 ppm) of O_3 produced an increase in serum protein esterase and in serum trypsin inhibitor. This latter effect may be a result of thickening of the small pulmonary arteries. The same exposure caused a significant decrease in albumin levels and an increase in alpha and gamma globulins (P'an and Jegier, 1971, 1976; P'an et al., 1972; Jegier, 1973). Chow et al. (1974) reported that the serum lysozyme level of rats increased significantly after 3 days of continuous exposure to O_3 but was not affected when the exposure was intermittent (8 hr/day, 7 days). The O_3 concentration in both studies was 1568 μg/m^3 (0.8 ppm) of O_3.

Short-term exposure to low concentrations of O_3 induced an immediate change in the serum creatine phosphokinase level in mice. In this study, the O_3 doses were expressed as the product of concentration and time. The C x T value for this effect ranged from 0.4 to 4.0 (Veninga et al., 1981).

A few of the hematological effects observed in animals (i.e., decrease in GSH and AChE activity and the formation of Heinz bodies) following exposure to O_3 have also been seen following _in vitro_ exposure of RBCs from humans (Freeman and Mudd, 1981; Menzel et al., 1975b; Verweij and Van Steveninck, 1981). A common effect observed by a number of investigators is that O_3 inhibits the membrane ATPase activity of RBCs (Koontz and Heath, 1979; Kesner et al., 1979; Kindya and Chan, 1976; Freeman et al., 1979; Verweij and Van Steveninck, 1980). It has been postulated that this inhibition of ATPase could be related to the spherocytosis and increased fragility of RBCs seen in animal and human cells.

Although these _in vitro_ data are useful in studying mechanisms of action, it is difficult to extrapolate these data to any effects observed in man. Not only is the method of exposure not physiological, but the actual concentration of O_3 reaching the RBC cannot be determined with any accuracy.

9.6.4.4 _Cytogenetic and Teratogenic Effects_. Uncertainty still exists regarding possible reproductive, teratogenic, and mutational effects of exposure to ozone. Based on various _in vitro_ data, a number of chromosomal effects of ozone have been described for isolated cultured cell lines, human lymphocytes, and microorganisms (Fetner, 1962; Hamelin et al., 1977a,b, Hamelin and Chung, 1975a,b, 1978; Scott and Lesher, 1963; Erdman and Hernandez, 1982; Guerrero et al., 1979; Dubeau and Chung, 1979, 1982). The interpretation, relevance, and predictive values of such studies to human health are questionable since (1) the concentrations used were many-fold greater than what is found in the ambient air (see Chapter 10); (2) extrapolation of in vitro exposure concentrations to human exposure dose is not yet possible; and (3) direct exposure of isolated cells to ozone is highly artifactual since it bypasses all the defenses of the host that would normally be functioning in protecting the individual from the inhaled gas. Furthermore, the direct exposure of isolated cells in vitro to ozone may result in chemical reactions between ozone and culture media that might not occur in vivo.

Important questions still exist regarding in vivo cytogenetic effects of ozone in rodents and humans. Zelac et al. (1971a,b) reported chromosomal abnormalities in peripheral leukocytes of hamsters exposed to O_3 (0.2 ppm). Combined exposures to ozone and radiation (227-233 rads) produced an additive effect on the number of chromosome breaks in peripheral leukocytes. These specific findings were not confirmed by Gooch et al. (1976) or by Tice et al. (1978), but sufficient differences in the various experimental protocols make a direct comparison difficult. The latter group did report significant increases in the number of chromatid deletions and achromatic lesions resulting from exposure to 0.43 ppm ozone.

Because the volume of air inspired during pregnancy is significantly enhanced, the pregnant animal may be at greater risk to low levels of ozone exposure. Early studies on the possible teratogenic effects of ozone have suggested that exposures as low as 0.2 ppm can reduce infant survival rate and cause unlimited incisor growth (Brinkman et al., 1964; Veninga, 1967). Kavlock et al. (1979, 1980) found that pregnant rats exposed to 1.0 and 1.49 ppm ozone showed a significant increase in embryo resorption rate, slower growth, slower development of righting reflexes, and delayed grooming and rearing behavior, but no increase in neonatal mortality was observed.

9.6.4.5 Other Extrapulmonary Effects. A series of studies was conducted to show that O_3 increases drug-induced sleeping time in a number of species of animals (Gardner et al., 1974; Graham, 1979; Graham et al., 1981, 1982a,b, 1983, 1985). At 1960 $\mu g/m^3$ (1.0 ppm), effects were observed after 1, 2, and 3 days of exposure. As the concentration of O_3 was reduced, increasing numbers of daily 3-hr exposures were required to produce a significant effect. At the lowest concentration studied (196 μg/m^3, 0.1 ppm), the increase was observed at days 15 and 16 of exposure. It appears that this effect is not specific to the strain of mouse or to the three species of animals tested, but it is sex-specific, with females being more susceptible. Recovery was complete within 24 hr after exposure. Although a number of mechanistic studies have been conducted, the reason for this effect on pentobarbital-induced sleeping time is not known. It has been hypothesized that some common aspect related to liver drug metabolism is quantitatively reduced (Graham et al., 1983).

Several investigators have attempted to elucidate the involvement of the endocrine system in O_3 toxicity. Most of these studies were designed to investigate the hypothesis that the survival rate of mice and rats exposed to lethal concentrations of O_3 could be increased by use of various thyroid blocking agents or by thyroidectomy. To follow up these findings, Clemons and Garcia (1980a,b) and Clemons and Wei (1984) investigated the effects of a 24-hr exposure to 1960 μg/m^3 (1.0 ppm) of O_3 on the hypothalamo-pituitary-thyroid system of rats. These three organs regulate the function of each other through various hormonal feedback mechanisms. Ozone caused decreases in serum concentration of thyroid stimulating hormone (TSH), in circulating thyroid hormones (T_3 and T_4) and in protein-bound iodine. No alterations were observed in many other hormone levels measured. Thyroidectomy prevented the effect of O_3 on TSH and T_4 and hypophysectomy prevented effects on T_4, unless the animals were supplemented with T_4 in their drinking water. The thyroid gland itself was altered (e.g., edema) by O_3. The authors hypothesyzed that O_3 alters serum binding of these hormones.

The extrapulmonary effects of ozone in experimental animals are summarized in Figure 9-9 and Table 9-33. Criteria used in developing the summary were presented in Section 9.6.1.

9.6.5 Interaction of Ozone With Other Pollutants

Combined exposure studies in laboratory animals have produced varied results, depending upon the pollutant combination evaluated and the measured variables. Additive and/or possibly synergistic effects of O_3 exposure in combination with NO_2 have been described for increased susceptibility to bacterial infection (Ehrlich et al., 1977, 1979; Ehrlich, 1980, 1983), morphological lesions (Freeman et al., 1974), and increased antioxidant metabolism (Mustafa et al., 1984). Additive or possibly synergistic effects from exposure

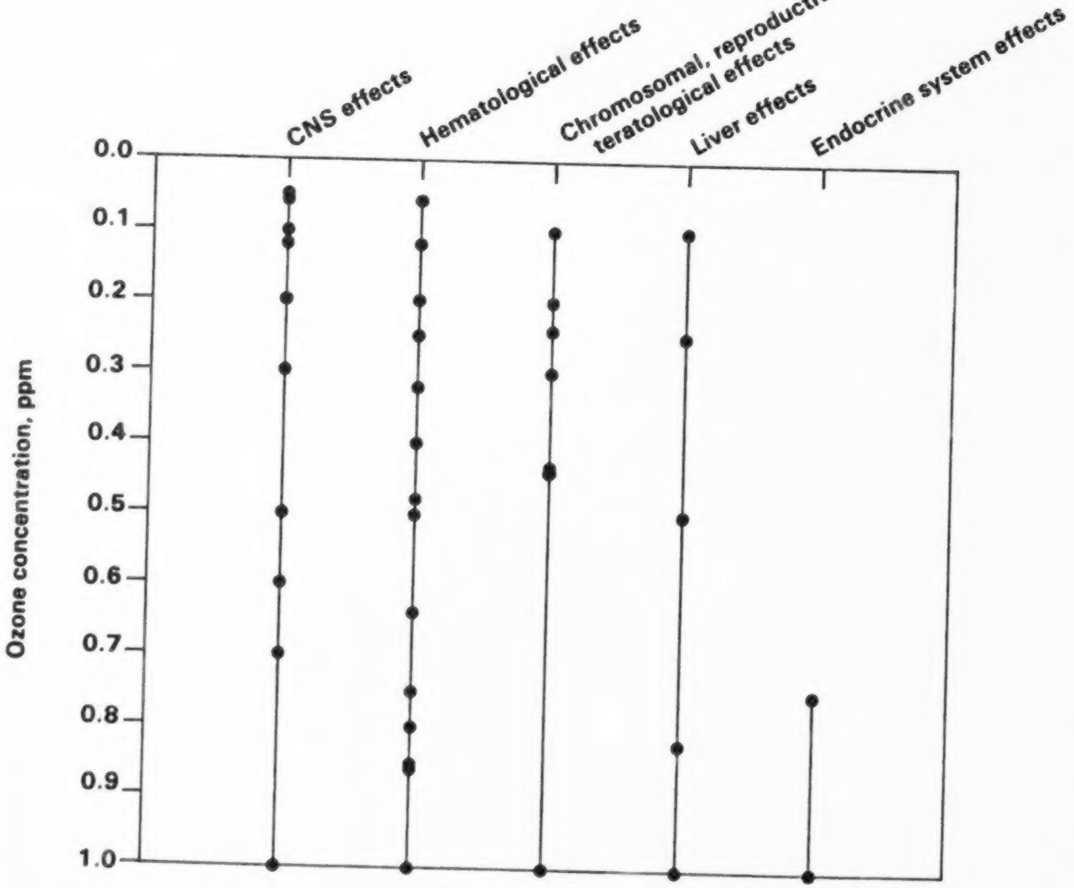

Figure 9-9. Summary of extrapulmonary effects of ozone in experimental animals. See Table 9-33 for reference citations of studies summarized here.

TABLE 9-33. SUMMARY TABLE: EXTRAPULMONARY EFFECTS OF OZONE IN EXPERIMENTAL ANIMALS

Effect/response	O_3 concentration, ppm	References
CNS effects	0.05, 0.5	Konigsberg and Bachman (1970)
	0.1 - 1.0	Weiss et al. (1981)
	0.12 - 1.0	Tepper et al. (1982)
	0.2, 0.3, 0.5, 0.7	Murphy et al. (1964)
	0.5	Tepper et al. (1983)
	0.5	Reynolds and Chaffee (1970)
	0.5	Xintaras et al. (1966)
	0.6	Peterson and Andrews (1963)
	1.0	Fletcher and Tappel (1973)
	1.0	Trams et al. (1972)
Hematological effects	0.06, 0.12, 0.48	Calabrese et al. (1983a)
	0.2	Brinkman et al. (1964)
	0.2, 1.0	Veninga (1967, 1970)
		Veninga et al. (1981)
	0.25, 0.32, 0.5	Moore et al. (1980; 1981a,b)
	0.4	Jegier (1973)
	0.4	P'an and Jegier (1972, 1976)
	0.5	Menzel et al. (1972)
	0.64	Larkin et al. (1983)
	0.75	Clark et al. (1978)
	0.8	Chow and Kaneko (1979)
	0.8	Chow et al. (1974)
	0.85	Menzel et al. (1975a)
	0.86	Schlipköter and Bruch (1973)
	1.0	Dorsey et al. (1983)
	1.0	Mizoguchi et al. (1973)
	1.0	Christiansen and Giese (1954)
Chromosomal, reproductive, teratological effects	0.1	Brinkman et al. (1964)
	0.2	Veninga (1967)
	0.24, 0.3	Zelac et al. (1971a)
	0.43	Tice et al. (1978)
	0.44	Kavlock et al. (1979)
	1.0	Kavlock et al. (1980)
Liver effects	0.1, 0.25, 0.5, 1.0	Graham (1979)
		Graham et al. (1981, 1982a,b)
	0.82	Veninga et al. (1981)
	1.0	Gardner et al. (1974)
Endocrine system effects	0.75	Atwal and Wilson (1974)
	0.75	Atwal et al. (1975)
	0.75	Atwal and Pemsingh (1981, 1984)
	0.75	Pemsingh and Atwal (1983)
	1.0	Clemons and Garcia (1980a,b)
	1.0	Clemons and Wei (1984)

to O_3 and H_2SO_4 have also been reported for host defense mechanisms (Gardner et al., 1977; Last and Cross, 1978; Grose et al., 1982), pulmonary sensitivity (Osebold et al. 1980), and collagen synthesis (Last et al., 1983), but not for morphology (Cavender et al., 1977; Moore and Schwartz, 1981). Mixtures of O_3 and $(NH_4)_2\ SO_4$ had synergistic effects on collagen synthesis and morphometry, including percentage of fibroblasts (Last et al., 1983, 1984a).

Combining O_3 with other particulate pollutants produces a variety of responses, depending on the endpoint measured. Mixtures of O_3, $Fe_2(SO_4)_3$, H_2SO_4, and $(NH_4)_2SO_4$ produced the same effect on clearance rate as exposure to O_3 alone. However, when measuring changes in host defenses, the combination of O_3 with NO_2 and $ZnSO_4$ or O_3 with SO_2 and $(NH_4)_2SO_4$ produced enhanced effects that can not be attributed to O_3 only.

However, since these issues are complex, they must be addressed experimentally using exposure regimens for combined pollutants that are more representative of ambient ratios of peak concentrations, frequency, duration, and time intervals between events.

The interactive effects of O_3 with other pollutants are summarized in Figure 9-10 and Table 9-34.

9.6.6 Effects of Other Photochemical Oxidants

There have been far too few controlled toxicological studies with the other oxidants to permit any sound scientific evaluation of their contribution to the toxic action of photochemical oxidant mixtures. When the effects seen after exposure to O_3 and PAN are examined and compared, it is obvious that the test animals must be exposed to concentrations of PAN much greater than those needed with O_3 to produce a similar effect on lethality, behavior modification, morphology, or significant alterations in host pulmonary defense system (Campbell et al., 1967; Dungworth et al., 1969; Thomas et al., 1979, 1981a). The concentrations of PAN required to produce these effects are many times greater than what has been measured in the atmosphere (0.047 ppm).

Similarly, most of the investigations reporting H_2O_2 toxicity have involved concentrations much higher than those found in the ambient air, or the investigations were conducted by using various in vitro techniques for exposure. Very limited information is available on the health significance of inhalation exposure to gaseous H_2O_2. Because H_2O_2 is highly soluble, it is generally assumed that it does not penetrate into the alveolar regions of the lung but is instead

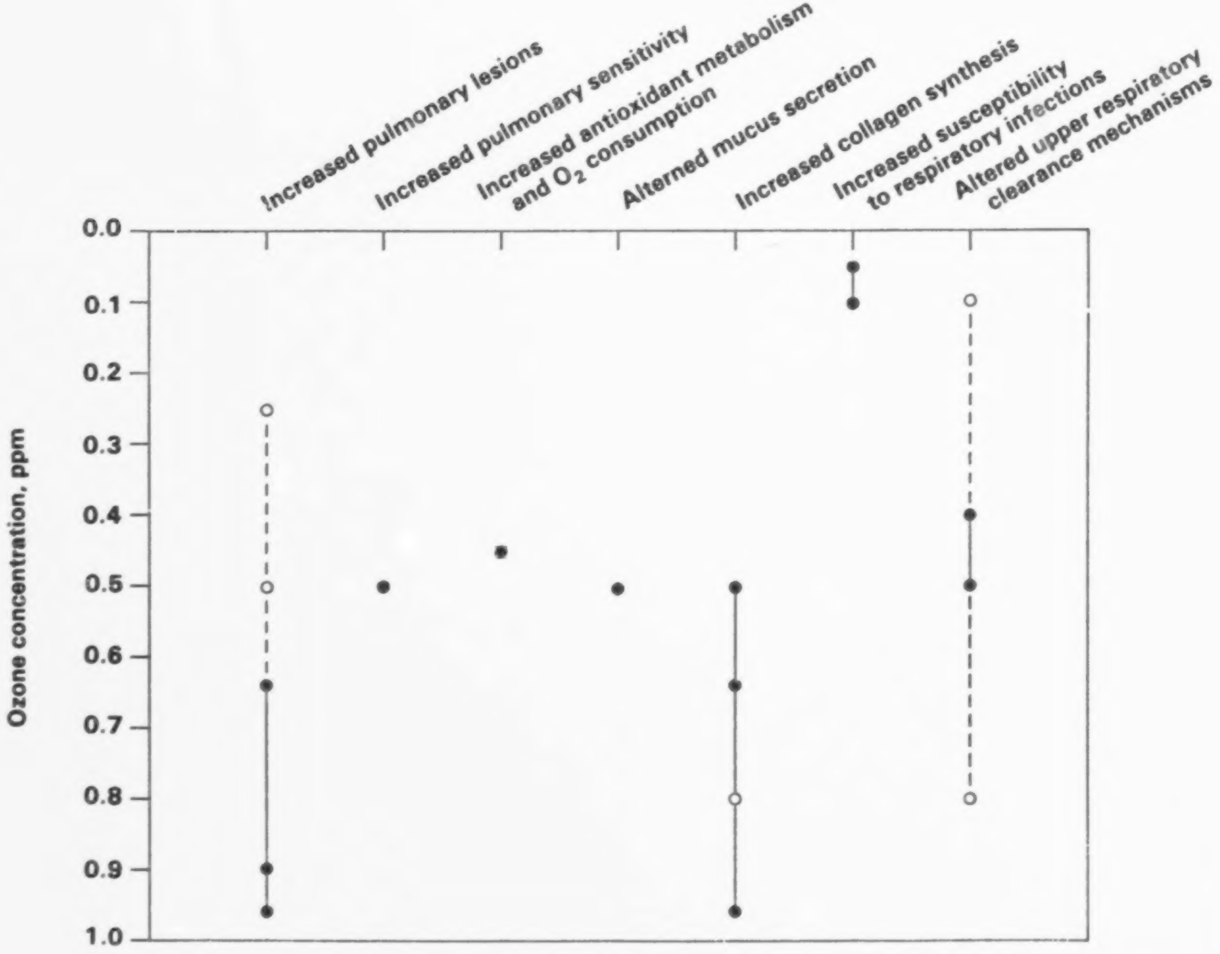

Figure 9-10. Summary of effects in experimental animals exposed to ozone combined with other pollutants. See Table 9-34 for reference citations of studies summarized here.

TABLE 9-34. SUMMARY TABLE: INTERACTION OF OZONE WITH OTHER POLLUTANTS IN EXPERIMENTAL ANIMALS

Effect/response	Pollutant concentrations	References
Increased pulmonary lesions	[0.25 ppm O_3 + 2.5 ppm NO_2]	Freeman et al. (1974)
	[0.5 ppm O_3 + 1 mg/m^3 H_2SO_4]	Moore and Schwartz (1981)
	[0.5 ppm O_3 + 10 mg/m^3 H_2SO_4	Cavender et al. (1978)
	0.64, 0.96 ppm O_3 + 5 mg/m^3 $(NH_4)_2 SO_4$	Last et al. (1984a)
	0.9 ppm O_3 + 0.9 ppm NO_2	Freeman et al. (1974)
	1.2 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$	Last et al. (1983)
Increased pulmonary sensitivity	0.5 ppm O_3 + 1 mg/m^3 H_2SO_4	Osebold et al. (1980)
Increased anti-oxidant metabolism and O_2 consumption	0.45 ppm O_3 + 4.8 ppm NO_2	Mustafa et al. (1984)
Altered mucus secretion	0.5 ppm O_3 + 1.1 mg/m^3 H_2SO_4	Last and Cross (1978); Last and Kaizu (1980)
Increased collagen synthesis	[0.5], [0.8], 1.5 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$	Last et al. (1983)
	0.5 ppm O_3 + 1 mg/m^3 H_2SO_4	Last et al. (1983)
	0.64, 0.96 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$	Last et al. (1984a)
Increased susceptibility to respiratory infections	0.05 ppm O_3 + 3760 μg/m^3 $(NH_4)_2SO_4$	Ehrlich et al. (1977, 1979); Ehrlich (1980)
	0.05 ppm O_3 + 100-400 μg/m^3 NO_2 + 1.5 mg/m^3 $ZnSO_4$	Ehrlich et al. (1983)
	0.1 ppm O_3 + 0.9 mg/m^3 H_2SO_4 (sequential exposure)	Gardner et al. (1977)
	0.1 ppm O_3 + 4.8 mg/m^3 H_2SO_4	Grose et al. (1982)
	0.1 ppm O_3 + 940 μg/m^3 NO_2	Ehrlich (1980)
	0.1 ppm O_3 + 13.2 mg/m^3 SO_2 + 1.0 mg/m^3 $(NH_4)_2SO_4$	Aranyi et al. (1983)

TABLE 9-34. SUMMARY TABLE: INTERACTION OF OZONE WITH OTHER POLLUTANTS (continued)

Effect/response	Pollutant concentrations	References
Altered upper respiratory clearance mechanisms	[0.1 ppm O_3 + 1.1 mg/m^3 H_2SO_4] (sequential exposure)	Grose et al. (1980)
	0.4 ppm O_3 + 7.0 ppm NO_2	Goldstein et al. (1974b)
	0.5 ppm O_3 + 3 mg/m^3 H_2SO_4	Last and Cross (1978)
	[0.8 ppm O_3 + 3.5 mg/m^3 $\{Fe_2(SO_4)_3 + H_2SO_4 + (NH_4)_2SO_4\}$]	Phalen et al. (1980)

deposited on the surface of the upper airways (Last et al., 1982). Unfortunately, there have not been studies designed to look for possible effects in this region of the respiratory tract.

A few *in vitro* studies have reported cytotoxic, genotoxic, and biochemical effects of H_2O_2 when using isolated cells or organs (Stewart et al., 1981; Bradley et al., 1979; Bradley and Erickson, 1981; Speit et al., 1982; MacRae and Stich, 1979). Although these studies can provide useful data for studying possible mechanisms of action, it is not yet possible to extrapolate these responses to those that might occur in the mammalian system.

Field and epidemiological studies have shown that human health effects from exposure to ambient mixtures of oxidants and other airborne pollutants can produce human health effects (Chapter 11). Few such studies have been conducted with laboratory animals, because testing and measuring of such mixtures is not only complicated, but extremely costly. In these studies, the investigators attempted to simulate the photochemical reaction products produced under natural conditions and to define the cause-effect relationship.

Exposure to complex mixtures of oxidants plus the various components found in UV-irradiated auto exhaust indicates that certain effects, such as histopathological changes, increase in susceptibility to infection, a variety of altered pulmonary functional activities were observed in this oxidant atmosphere which was not reported in the nonirradiated exhaust (Murphy et al., 1963; Murphy, 1964; Nakajima et al., 1972; Hueter et al., 1966). Certain other biological responses were observed in both treatment groups, including a decrease in spontaneous activity, a decrease in infant survival rate, fertility, and certain pulmonary functional abnormalities (Hueter et al., 1966; Boche and Quilligan, 1960; Lewis et al., 1967).

Dogs exposed to UV-irradiated auto exhaust containing oxidants either with or without SO_x showed significant pulmonary functional abnormalities that had relatively good correlation with structural changes (Hyde et al., 1978; Gillespie, 1980; Lewis et al., 1974). There were no significant differences in the magnitude of the response in these two treatment groups, indicating that oxidant gases and SO_x did not interact in any synergistic or additive manner.

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APPENDIX A: GLOSSARY OF PULMONARY TERMS AND SYMBOLS*

Acetylcholine (ACh): A naturally occurring substance in the body having important parasympathetic effects; often used as a bronchoconstrictor.

Aerosol: Solid particles or liquid droplets that are dispersed or suspended in a gas, ranging in size from 10^{-4} to 10^{2} micrometers (μm).

Air spaces: All alveolar ducts, alveolar sacs, and alveoli. To be contrasted with AIRWAYS.

Airway conductance (Gaw): Reciprocal of airway resistance. Gaw = (1/Raw).

Airway resistance (Raw): The (frictional) resistance to airflow afforded by the airways between the airway opening at the mouth and the alveoli.

Airways: All passageways of the respiratory tract from mouth or nares down to and including respiratory bronchioles. To be contrasted with AIR SPACES.

Allergen: A material that, as a result of coming into contact with appropriate tissues of an animal body, induces a state of allergy or hypersensitivity; generally associated with idiosyncratic hypersensitivities.

Alveolar-arterial oxygen pressure difference [$P(A-a)O_2$]: The difference in partial pressure of O_2 in the alveolar gas spaces and that in the systemic arterial blood, measured in torr.

Alveolar-capillary membrane: A fine membrane (0.2 to 0.4 μm) separating alveolus from capillary; composed of epithelial cells lining the alveolus, a thin layer of connective tissue, and a layer of capillary endothelial cells.

Alveolar carbon dioxide pressure (P_ACO_2): Partial pressure of carbon dioxide in the air contained in the lung alveoli.

Alveolar oxygen partial pressure (P_AO_2): Partial pressure of oxygen in the air contained in the alveoli of the lungs.

Alveolar septum (pl. septa): A thin tissue partition between two adjacent pulmonary alveoli, consisting of a close-meshed capillary network and interstitium covered on both surfaces by alveolar epithelial cells.

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Alveolitis: (interstitial pneumonia): Inflammation of the lung distal to the terminal non-respiratory bronchiole. Unless otherwise indicated, it is assumed that the condition is diffuse. Arbitrarily, the term is not used to refer to exudate in air spaces resulting from bacterial infection of the lung.

Alveolus: Hexagonal or spherical air cells of the lungs. The majority of alveoli arise from the alveolar ducts which are lined with the alveoli. An alveolus is an ultimate respiratory unit where the gas exchange takes place.

Anatomical dead space ($V_{D\ anat}$): Volume of the conducting airways down to the level where, during air breathing, gas exchange with blood can occur, a region probably situated at the entrance of the alveolar ducts.

Arterial oxygen saturation (SaO_2): Percent saturation of dissolved oxygen in arterial blood.

Arterial partial pressure of carbon dioxide ($PaCO_2$): Partial pressure of dissolved carbon dioxide in arterial blood.

Arterial partial pressure of oxygen (PaO_2): Partial pressure of dissolved oxygen in arterial blood.

Asthma: A disease characterized by an increased responsiveness of the airways to various stimuli and manifested by slowing of forced expiration which changes in severity either spontaneously or as a result of therapy. The term asthma may be modified by words or phrases indicating its etiology, factors provoking attacks, or its duration.

Atelectasis: State of collapse of air spaces with elimination of the gas phase.

ATPS condition (ATPS): Ambient temperature and pressure, saturated with water vapor. These are the conditions existing in a water spirometer.

Atropine: A poisonous white crystalline alkaloid, $C_{17}H_{23}NO_3$, from belladonna and related plants, used to relieve spasms of smooth muscles. It is an anticholinergic agent.

Breathing pattern: A general term designating the characteristics of the ventilatory activity, e.g., tidal volume, frequency of breathing, and shape of the volume time curve.

Breuer-Hering reflexes (Hering-Breuer reflexes): Ventilatory reflexes originating in the lungs. The reflex arcs are formed by the pulmonary mechanoreceptors, the vagal afferent fibers, the respiratory centers, the medullospinal pathway, the motor neurons, and the respiratory muscles. The afferent link informs the respiratory centers of the volume state or of the rate of change of volume of the lungs. Three types of Breuer-Hering reflexes have been described: 1) an inflation reflex in which lung inflation tends to inhibit inspiration and stimulate expiration; 2) a deflation reflex in which lung deflation tends to inhibit expiration and stimulate inspiration; and 3) a "paradoxical reflex," described but largely disregarded by Breuer and Hering, in which sudden inflation may stimulate inspiratory muscles.

Bronchiole: One of the finer subdivisions of the airways, less than 1 mm in diameter, and having no cartilage in its wall.

Bronchiolitis: Inflammation of the bronchioles which may be acute or chronic. If the etiology is known, it should be stated. If permanent occlusion of the lumens is present, the term bronchiolitis obliterans may be used.

Bronchitis: A non-neoplastic disorder of structure or function of the bronchi resulting from infectious or noninfectious irritation. The term bronchitis should be modified by appropriate words or phrases to indicate its etiology, its chronicity, the presence of associated airways dysfunction, or type of anatomic change. The term chronic bronchitis, when unqualified, refers to a condition associated with prolonged exposure to nonspecific bronchial irritants and accompanied by mucous hypersecretion and certain structural alterations in the bronchi. Anatomic changes may include hypertrophy of the mucous-secreting apparatus and epithelial metaplasia, as well as more classic evidences of inflammation. In epidemiologic studies, the presence of cough or sputum production on most days for at least three months of the year has sometimes been accepted as a criterion for the diagnosis.

Bronchoconstrictor: An agent that causes a reduction in the caliber (diameter) of airways.

Bronchodilator: An agent that causes an increase in the caliber (diameter) of airways.

Bronchus: One of the subdivisions of the trachea serving to convey air to and from the lungs. The trachea divides into right and left main bronchi which in turn form lobar, segmental, and subsegmental bronchi.

BTPS conditions (BTPS): Body temperature, barometric pressure, and saturated with water vapor. These are the conditions existing in the gas phase of the lungs. For man the normal temperature is taken as 37°C, the pressure as the barometric pressure, and the partial pressure of water vapor as 47 torr.

Carbachol: A parasympathetic stimulant (carbamoylcholine chloride, $C_6H_{15}ClN_2O_2$) that produces constriction of the bronchial smooth muscles.

Carbon dioxide production ($\dot{V}CO_2$): Rate of carbon dioxide production by organisms, tissues, or cells. Common units: ml CO_2 (STPD)/kg·min.

Carbon monoxide (CO): An odorless, colorless, toxic gas formed by incomplete combustion, with a strong affinity for hemoglobin and cytochrome; it reduces oxygen absorption capacity, transport, and utilization.

Carboxyhemoglobin (COHb): Hemoglobin in which the iron is associated with carbon monoxide. The affinity of hemoglobin for CO is about 300 times greater than for O_2.

Chronic obstructive lung disease (COLD): This term refers to diseases of uncertain etiology characterized by persistent slowing of airflow during forced expiration. It is recommended that a more specific term, such as chronic obstructive bronchitis or chronic obstructive emphysema, be used whenever possible. Synonymous with chronic obstructive pulmonary disease (COPD).

Closing capacity (CC): Closing volume plus residual volume, often expressed as a ratio of TLC, i.e. (CC/TLC%).

Closing volume (CV): The volume exhaled after the expired gas concentration is inflected from an alveolar plateau during a controlled breathing maneuver. Since the value obtained is dependent on the specific test technique, the method used must be designated in the text, and when necessary, specified by a qualifying symbol. Closing volume is often expressed as a ratio of the VC, i.e. (CV/VC%).

Collateral resistance (R_{coll}): Resistance to flow through indirect pathways. See COLLATERAL VENTILATION and RESISTANCE.

Collateral ventilation: Ventilation of air spaces via indirect pathways, e.g., through pores in alveolar septa, or anastomosing respiratory bronchioles.

Compliance (C_L,C_{st}): A measure of distensibility. Pulmonary compliance is given by the slope of a static volume-pressure curve at a point, or the linear approximation of a nearly straight portion of such a curve, expressed in liters/cm H_2O or ml/cm H_2O. Since the static volume-pressure characteristics of lungs are nonlinear (static compliance decreases as lung volume increases) and vary according to the previous volume history (static compliance at a given volume increases immediately after full inflation and decreases following deflation), careful specification of the conditions of measurement are necessary. Absolute values also depend on organ size. See also DYNAMIC COMPLIANCE.

Conductance (G): The reciprocal of RESISTANCE. See AIRWAY CONDUCTANCE.

Diffusing capacity of the lung (D_L, D_LO_2, D_LCO_2, D_LCO): Amount of gas (O_2, CO, CO_2) commonly expressed as ml gas (STPD) diffusing between alveolar gas and pulmonary capillary blood per torr mean gas pressure difference per min, i.e., ml O_2/(min-torr). Synonymous with transfer factor and diffusion factor.

Dynamic compliance (C_{dyn}): The ratio of the tidal volume to the change in intrapleural pressure between the points of zero flow at the extremes of tidal volume in liters/cm H_2O or ml/cm H_2O. Since at the points of zero airflow at the extremes of tidal volume, volume acceleration is usually other than zero, and since, particularly in abnormal states, flow may still be taking place within lungs between regions which are exchanging volume, dynamic compliance may differ from static compliance, the latter pertaining to condition of zero volume acceleration and zero gas flow throughout the lungs. In normal lungs at ordinary volumes and respiratory frequencies, static and dynamic compliance are the same.

Elastance (E): The reciprocal of COMPLIANCE; expressed in cm H_2O/liter or cm H_2O/ml.

Electrocardiogram (ECG, EKG): The graphic record of the electrical currents that are associated with the heart's contraction and relaxation.

Emphysema: A condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by the destruction of their walls, and without obvious fibrosis.

Expiratory reserve volume (ERV): The maximal volume of air exhaled from the end-expiratory level.

FEV_t/FVC: A ratio of timed (t = 0.5, 1, 2, 3 s) forced expiratory volume (FEV_t) to forced vital capacity (FVC). The ratio is often expressed in percent 100 x FEV_t/FVC. It is an index of airway obstruction.

Flow volume curve: Graph of instantaneous forced expiratory flow recorded at the mouth, against corresponding lung volume. When recorded over the full vital capacity, the curve includes maximum expiratory flow rates at all lung volumes in the VC range and is called a maximum expiratory flow-volume curve (MEFV). A partial expiratory flow-volume curve (PEFV) is one which describes maximum expiratory flow rate over a portion of the vital capacity only.

Forced expiratory flow (FEFx): Related to some portion of the FVC curve. Modifiers refer to the amount of the FVC already exhaled when the measurement is made. For example:

$FEF_{75\%}$ = instantaneous forced expiratory flow after 75% of the FVC has been exhaled.

$FEF_{200-1200}$ = mean forced expiratory flow between 200 ml and 1200 ml of the FVC (formerly called the maximum expiratory flow rate (MEFR).

$FEF_{25-75\%}$ = mean forced expiratory flow during the middle half of the FVC [formerly called the maximum mid-expiratory flow rate (MMFR)].

FEF_{max} = the maximal forced expiratory flow achieved during an FVC.

Forced expiratory volume (FEV): Denotes the volume of gas which is exhaled in a given time interval during the execution of a forced vital capacity. Conventionally, the times used are 0.5, 0.75, or 1 sec, symbolized $FEV_{0.5}$, $FEV_{0.75}$, $FEV_{1.0}$. These values are often expressed as a percent of the forced vital capacity, e.g. ($FEV_{1.0}$/VC) X 100.

Forced inspiratory vital capacity (FIVC): The maximal volume of air inspired with a maximally forced effort from a position of maximal expiration.

Forced vital capacity (FVC): Vital capacity performed with a maximally forced expiratory effort.

Functional residual capacity (FRC): The sum of RV and ERV (the volume of air remaining in the lungs at the end-expiratory position). The method of measurement should be indicated as with RV.

Gas exchange: Movement of oxygen from the alveoli into the pulmonary capillary blood as carbon dioxide enters the alveoli from the blood. In broader terms, the exchange of gases between alveoli and lung capillaries.

Gas exchange ratio (R): See RESPIRATORY QUOTIENT.

Gas trapping: Trapping of gas behind small airways that were opened during inspiration but closed during forceful expiration. It is a volume difference between FVC and VC.

Hematocrit (Hct): The percentage of the volume of red blood cells in whole blood.

Hemoglobin (Hb): A hemoprotein naturally occurring in most vertebrate blood, consisting of four polypeptide chains (the globulin) to each of which there is attached a heme group. The heme is made of four pyrrole rings and a divalent iron (Fe^{2+}-protoporphyrin) which combines reversibly with molecular oxygen.

Histamine: A depressor amine derived from the amino acid histidine and found in all body tissues, with the highest concentration in the lung; a powerful stimulant of gastric secretion, a constrictor of bronchial smooth muscle, and a vasodilator that causes a fall in blood pressure.

Hypoxemia: A state in which the oxygen pressure and/or concentration in arterial and/or venous blood is lower than its normal value at sea level. Normal oxygen pressures at sea level are 85-100 torr in arterial blood and 37-44 torr in mixed venous blood. In adult humans the normal oxygen concentration is 17-23 ml O_2/100 ml arterial blood; in mixed venous blood at rest it is 13-18 ml O_2/100 ml blood.

Hypoxia: Any state in which the oxygen in the lung, blood, and/or tissues is abnormally low compared with that of normal resting man breathing air at sea level. If the P_{O2} is low in the environment, whether because of decreased barometric pressure or decreased fractional concentration of O_2, the condition is termed environmental hypoxia. Hypoxia when referring to the blood is termed hypoxemia. Tissues are said to be hypoxic when their P_{O2} is low, even if there is no arterial hypoxemia, as in "stagnant hypoxia" which occurs when the local circulation is low compared to the local metabolism.

Inspiratory capacity (IC): The sum of IRV and TV.

Inspiratory reserve volume (IRV): The maximal volume of air inhaled from the end-inspiratory level.

Inspiratory vital capacity (IVC): The maximum volume of air inhaled from the point of maximum expiration.

Kilogram-meter/min (kg-m/min): The work performed each min to move a mass of 1 kg through a vertical distance of 1 m against the force of gravity. Synonymous with kilopond-meter/min.

Lung volume (V_L): Actual volume of the lung, including the volume of the conducting airways.

Maximal aerobic capacity (max $\dot{V}O_2$): The rate of oxygen uptake by the body during repetitive maximal respiratory effort. Synonymous with maximal oxygen consumption.

Maximum breathing capacity (MBC): Maximal volume of air which can be breathed per minute by a subject breathing as quickly and as deeply as possible. This tiring lung function test is usually limited to 12-20 sec, but given in liters (BTPS)/min. Synonymous with maximum voluntary ventilation (MVV).

Maximum expiratory flow ($\dot{V}_{max\ x}$): Forced expiratory flow, related to the total lung capacity or the actual volume of the lung at which the measurement is made. Modifiers refer to the amount of lung volume remaining when the measurement is made. For example:

$\dot{V}_{max\ 75\%}$ = instantaneous forced expiratory flow when the lung is at 75% of its TLC.

$\dot{V}_{max\ 3.0}$ = instantaneous forced expiratory flow when the lung volume is 3.0 liters

Maximum expiratory flow rate (MEFR): Synonymous with $FEF_{200-1200}$.

Maximum mid-expiratory flow rate (MMFR or MMEF): Synonymous with $FEF_{25-75\%}$.

Maximum ventilation (max $\dot{V}_E$): The volume of air breathed in one minute during repetitive maximal respiratory effort. Synonymous with maximum ventilatory minute volume.

Maximum voluntary ventilation (MVV): The volume of air breathed by a subject during voluntary maximum hyperventilation lasting a specific period of time. Synonymous with maximum breathing capacity (MBC).

Methemoglobin (MetHb): Hemoglobin in which iron is in the ferric state. Because the iron is oxidized, methemoglobin is incapable of oxygen transport. Methemoglobins are formed by various drugs and occur under pathological conditions. Many methods for hemoglobin measurements utilize methemoglobin (chlorhemiglobin, cyanhemiglobin).

Minute ventilation ($\dot{V}_E$): Volume of air breathed in one minute. It is a product of tidal volume (V_T) and breathing frequency (f_B). See VENTILATION.

Minute volume: Synonymous with minute ventilation.

Mucociliary transport: The process by which mucus is transported, by ciliary action, from the lungs.

Mucus: The clear, viscid secretion of mucous membranes, consisting of mucin, epithelial cells, leukocytes, and various inorganic salts suspended in water.

Nasopharyngeal: Relating to the nose or the nasal cavity and the pharynx (throat).

Nitrogen oxides: Compounds of N and O in ambient air; i.e., nitric oxide (NO) and others with a higher oxidation state of N, of which NO_2 is the most important toxicologically.

Nitrogen washout (ΔN_2, dN_2): The curve obtained by plotting the fractional concentration of N_2 in expired alveolar gas vs. time, for a subject switched from breathing ambient air to an inspired mixture of pure O_2. A progressive decrease of N_2 concentration ensues which may be analyzed into two or more exponential components. Normally, after 4 min of pure O_2 breathing the fractional N_2 concentration in expired alveolar gas is down to less than 2%.

Normoxia: A state in which the ambient oxygen pressure is approximately 150 ± 10 torr (i.e., the partial pressure of oxygen in air at sea level).

Oxidant: A chemical compound that has the ability to remove, accept, or share electrons from another chemical species, thereby oxidizing it.

Oxygen consumption ($\dot{V}O_2$, $\dot{Q}O_2$): Rate of oxygen uptake of organisms, tissues, or cells. Common units: ml O_2 (STPD)/(kg·min) or ml O_2 (STPD)/(kg·hr). For whole organisms the oxygen consumption is commonly expressed per unit surface area or some power of the body weight. For tissue samples or isolated cells $\dot{Q}_{O2}$ = µl O_2/hr per mg dry weight.

Oxygen saturation (SO_2): The amount of oxygen combined with hemoglobin, expressed as a percentage of the oxygen capacity of that hemoglobin. In arterial blood, SaO_2.

Oxygen uptake ($\dot{V}O_2$): Amount of oxygen taken up by the body from the environment, by the blood from the alveolar gas, or by an organ or tissue from the blood. When this amount of oxygen is expressed per unit of time one deals with an "oxygen uptake rate." "Oxygen consumption" refers more specifically to the oxygen uptake rate by all tissues of the body and is equal to the oxygen uptake rate of the organism only when the O_2 stores are constant.

Particulates: Fine solid particles such as dust, smoke, fumes, or smog, found in the air or in emissions.

Pathogen: Any virus, microorganism, or etiologic agent causing disease.

Peak expiratory flow (PEF): The highest forced expiratory flow measured with a peak flow meter.

Peroxyacetyl nitrate (PAN): Pollutant created by action of UV component of sunlight on hydrocarbons and NO_x in the air; an ingredient of photochemical smog.

Physiological dead space (V_D): Calculated volume which accounts for the difference between the pressures of CO_2 in expired and alveolar gas (or arterial blood). Physiological dead space reflects the combination of anatomical dead space and alveolar dead space, the volume of the latter increasing with the importance of the nonuniformity of the ventilation/perfusion ratio in the lung.

Plethysmograph: A rigid chamber placed around a living structure for the purpose of measuring changes in the volume of the structure. In respiratory measurements, the entire body is ordinarily enclosed ("body plethysmograph") and the plethysmograph is used to measure changes in volume of gas in the system produced 1) by solution and volatilization (e.g., uptake of foreign gases into the blood), 2) by changes in pressure or temperature (e.g., gas compression in the lungs, expansion of gas upon passing into the warm, moist lungs), or 3) by breathing through a tube to the outside. Three types of plethysmograph are used: a) pressure, b) volume, and c) pressure-volume. In type a, the body chambers have fixed volumes and volume changes are measured in terms of pressure change secondary to gas compression (inside the chamber, outside the body). In type b, the body chambers serve essentially as conduits between the body surface and devices (spirometers or integrating flowmeters) which measure gas displacements. Type c combines a and b by appropriate summing of chamber pressure and volume displacements.

Pneumotachograph: A device for measuring instantaneous gas flow rates in breathing by recording the pressure drop across a fixed flow resistance of known pressure-flow characteristics, commonly connected to the airway by means of a mouthpiece, face mask, or cannula. The flow resistance usually consists either of parallel capillary tubes (Fleisch type) or of fine-meshed screen (Silverman-Lilly type).

Pulmonary alveolar proteinosis: A chronic or recurrent disease characterized by the filling of alveoli with an insoluble exudate, usually poor in cells, rich in lipids and proteins, and accompanied by minimal histologic alteration of the alveolar walls.

Pulmonary edema: An accumulation of excessive amounts of fluid in the lung extravascular tissue and air spaces.

Pulmonary emphysema: An abnormal, permanent enlargement of the air spaces distal to the terminal nonrespiratory bronchiole, accompanied by destructive changes of the alveolar walls and without obvious fibrosis. The term emphysema may be modified by words or phrases to indicate its etiology, its anatomic subtype, or any associated airways dysfunction.

Residual volume (RV): That volume of air remaining in the lungs after maximal exhalation. The method of measurement should be indicated in the text or, when necessary, by appropriate qualifying symbols.

Resistance flow (R): The ratio of the flow-resistive components of pressure to simultaneous flow, in cm H_2O/liter per sec. Flow-resistive components of pressure are obtained by subtracting any elastic or inertial components, proportional respectively to volume and volume acceleration. Most flow resistances in the respiratory system are nonlinear, varying with the magnitude and direction of flow, with lung volume and lung volume history, and possibly with volume acceleration. Accordingly, careful specification of the conditions of measurement is necessary; see AIRWAY RESISTANCE, TISSUE RESISTANCE, TOTAL PULMONARY RESISTANCE, COLLATERAL RESISTANCE.

Respiratory cycle: A respiratory cycle is constituted by the inspiration followed by the expiration of a given volume of gas, called tidal volume. The duration of the respiratory cycle is the respiratory or ventilatory period, whose reciprocal is the ventilatory frequency.

Respiratory exchange ratio: See RESPIRATORY QUOTIENT.

Respiratory frequency (f_R): The number of breathing cycles per unit of time. Synonymous with breathing frequency (f_B).

Respiratory quotient (RQ, R): Quotient of the volume of CO_2 produced divided by the volume of O_2 consumed by an organism, an organ, or a tissue during a given period of time. Respiratory quotients are measured by comparing the composition of an incoming and an outgoing medium, e.g., inspired and expired gas, inspired gas and alveolar gas, or arterial and venous blood. Sometimes the phrase "respiratory exchange ratio" is used to designate the ratio of CO_2 output to the O_2 uptake by the lungs, "respiratory quotient" being restricted to the actual metabolic CO_2 output and O_2 uptake by the tissues. With this definition, respiratory quotient and respiratory exchange ratio are identical in the steady state, a condition which implies constancy of the O_2 and CO_2 stores.

Shunt: Vascular connection between circulatory pathways so that venous blood is diverted into vessels containing arterialized blood (right-to-left shunt, venous admixture) or vice versa (left-to-right shunt). Right-to-left shunt within the lung, heart, or large vessels due to malformations are more important in respiratory physiology. Flow from left to right through a shunt should be marked with a negative sign.

Specific airway conductance (SGaw): Airway conductance divided by the lung volume at which it was measured, i.e., normalized airway conductance. SGaw = Gaw/TGV.

Specific airway resistance (SRaw): Airway resistance multiplied by the volume at which it was measured. SRaw = Raw x TGV.

Spirograph: Mechanical device, including bellows or other scaled, moving part, which collects and stores gases and provides a graphical record of volume changes. See BREATHING PATTERN, RESPIRATORY CYCLE.

Spirometer: An apparatus similar to a spirograph but without recording facility.

Static lung compliance (C_{Lst}): Lung compliance measured at zero flow (breath-holding) over linear portion of the volume-pressure curve above FRC. See COMPLIANCE.

Static transpulmonary pressure (P_{st}): Transpulmonary pressure measured at a specified lung volume; e.g., P_{st}TLC is static recoil pressure measured at TLC (maximum recoil pressure).

Sulfur dioxide (SO_2): Colorless gas with pungent odor, released primarily from burning of fossil fuels, such as coal, containing sulfur.

STPD conditions (STPD): Standard temperature and pressure, dry. These are the conditions of a volume of gas at 0°C, at 760 torr, without water vapor. A STPD volume of a given gas contains a known number of moles of that gas.

Surfactant, pulmonary: Protein-phospholipid (mainly dipalmitoyl lecithin) complex which lines alveoli (and possibly small airways) and accounts for the low surface tension which makes air space (and airway) patency possible at low transpulmonary pressures.

Synergism: A relationship in which the combined action or effect of two or more components is greater than the sum of effects when the components act separately.

Thoracic gas volume (TGV): Volume of communicating and trapped gas in the lungs measured by body plethysmography at specific lung volumes. In normal subjects, TGV determined at end expiratory level corresponds to FRC.

Tidal volume (TV): That volume of air inhaled or exhaled with each breath during quiet breathing, used only to indicate a subdivision of lung volume. When tidal volume is used in gas exchange formulations, the symbol V_T should be used.

Tissue resistance (R_{ti}): Frictional resistance of the pulmonary and thoracic tissues.

Torr: A unit of pressure equal to 1,333.22 dynes/cm^2 or 1.33322 millibars. The torr is equal to the pressure required to support a column of mercury 1 mm high when the mercury is of standard density and subjected to standard acceleration. These standard conditions are met at 0°C and 45° latitude, where the acceleration of gravity is 980.6 cm/sec^2. In reading a mercury barometer at other temperatures and latitudes, corrections, which commonly exceed 2 torr, must be introduced for these terms and for the thermal expansion of the measuring scale used. The torr is synonymous with pressure unit mm Hg.

Total lung capacity (TLC): The sum of all volume compartments or the volume of air in the lungs after maximal inspiration. The method of measurement should be indicated, as with RV.

Total pulmonary resistance (R_L): Resistance measured by relating flow-dependent transpulmonary pressure to airflow at the mouth. Represents the total (frictional) resistance of the lung tissue (R_{ti}) and the airways (Raw). $R_L = R_{aw} + R_{ti}$.

Trachea: Commonly known as the windpipe; a cartilaginous air tube extending from the larynx (voice box) into the thorax (chest) where it divides into left and right branches.

Transpulmonary pressure (P_L): Pressure difference between airway opening (mouth, nares, or cannula opening) and the visceral pleural surface, in cm H_2O. Transpulmonary in the sense used includes extrapulmonary structures, e.g., trachea and extrathoracic airways. This usage has come about for want of an anatomic term which includes all of the airways and the lungs together.

Ventilation: Physiological process by which gas is renewed in the lungs. The word ventilation sometimes designates ventilatory flow rate (or ventilatory minute volume) which is the product of the tidal volume by the ventilatory frequency. Conditions are usually indicated as modifiers; i.e.,

$\dot{V}_E$ = Expired volume per minute (BTPS),
and
$\dot{V}_I$ = Inspired volume per minute (BTPS).

Ventilation is often referred to as "total ventilation" to distinguish it from "alveolar ventilation" (see VENTILATION, ALVEOLAR).

Ventilation, alveolar ($\dot{V}_A$): Physiological process by which alveolar gas is completely removed and replaced with fresh gas. Alveolar ventilation is less than total ventilation because when a tidal volume of gas leaves the alveolar spaces, the last part does not get expelled from the body but occupies the dead space, to be reinspired with the next inspiration. Thus the volume of alveolar gas actually expelled completely is equal to the tidal volume minus the volume of the dead space. This truly complete expiration volume times the ventilatory frequency constitutes the alveolar ventilation.

Ventilation, dead-space ($\dot{V}_D$): Ventilation per minute of the physiologic dead space (wasted ventilation), BTPS, defined by the following equation:

$$\dot{V}_D = \dot{V}_E(PaCO_2 - P_ECO_2)/(PaCO_2 - P_ICO_2)$$

Ventilation/perfusion ratio ($\dot{V}_A/\dot{Q}$): Ratio of the alveolar ventilation to the blood perfusion volume flow through the pulmonary parenchyma. This ratio is a fundamental determinant of the O_2 and CO_2 pressure of the alveolar gas and of the end-capillary blood. Throughout the lungs the local ventilation/perfusion ratios vary, and consequently the local alveolar gas and end-capillary blood compositions also vary.

Vital capacity (VC): The maximum volume of air exhaled from the point of maximum inspiration.

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